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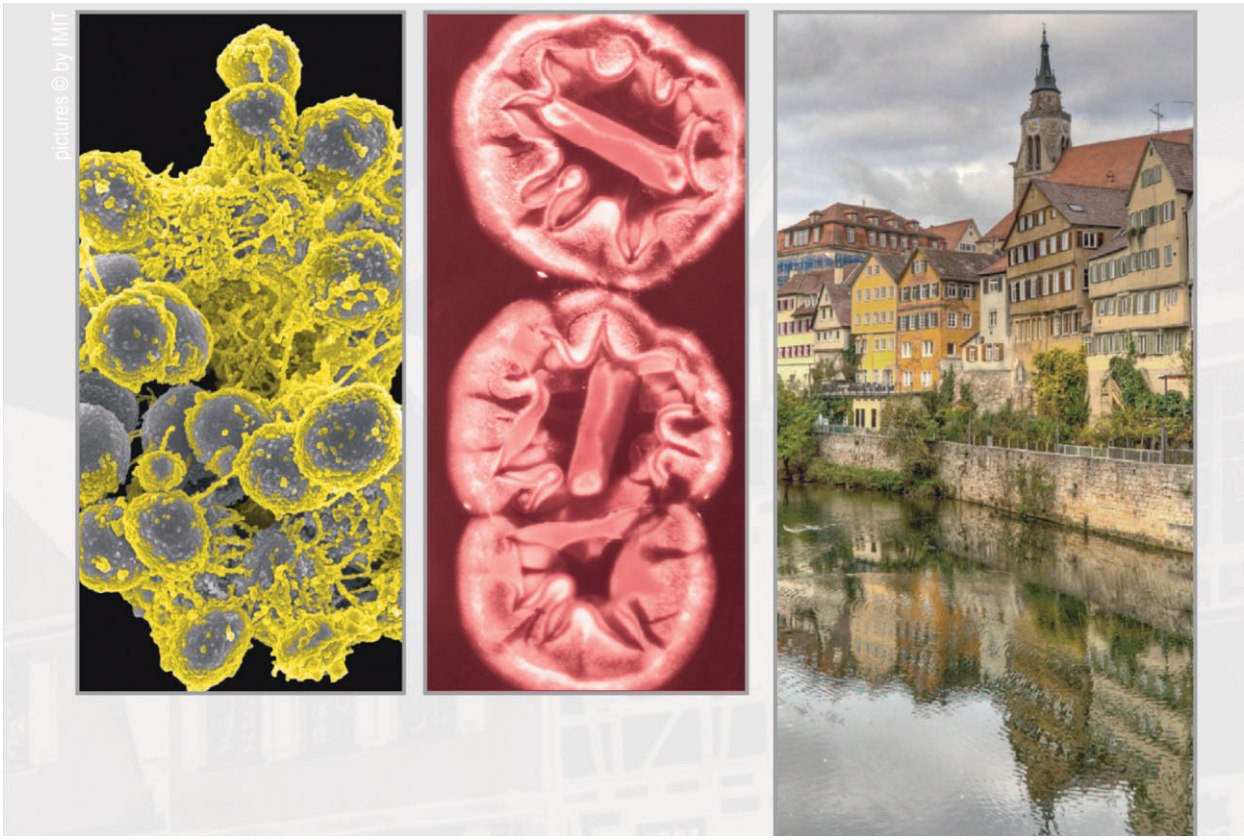

2012

Sonderausgabe

Tagungsband zur

VAAM-Jahrestagung 2012

18.–21. März in Tübingen

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Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

Tagungsband zur VAAM-Jahrestagung 2012

18. bis 21. März in Tübingen

Conference President: Prof. Dr. Wolfgang Wohlleben

Scientific Committee:
Eberhard Karls Universität Tübingen,
Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen (IMIT):
Medizinische Mikrobiologie und Hygiene: Ingo Autenrieth; Zelluläre und Molekulare Mikrobiologie: Andreas Peschel; Mikrobiologie/Biotechnologie: Wolfgang Wohlleben; Mikrobiologie/Organismische Interaktionen: Karl Forchhammer; Mikrobielle Genetik: Friedrich Götz; Physiologische Ökologie der Pflanzen: Rüdiger Hampp; *Pharmazeutische Biologie*: Lutz Heide; *Angewandte Geowissenschaften*: Andreas Kappler; *Max-Planck-Institut für Entwicklungsbiologie:* Volkmar Braun und Andrei Lupas.

Zum Titelbild:

From left to right:
S. aureus: Zellen in einer Biofilmmatrix in Falschfarben (Michael Otto, NIH, Washington DC).
Colonies of *Streptomyces spec.* TÜ6152 on agar plate (Hans-Peter Fiedler, Universität Tübingen)
Scenic view of the Neckar front (Tilmann Weber, Universtät Tübingen)

Grußwort der Stadt Tübingen

Liebe Teilnehmerinnen und Teilnehmer,

„Tübingen hat keine Universität, Tübingen ist eine Universität." Dieses Zitat von Walter Jens beschreibt die Stadt am Neckar auch heute noch treffend. Seit Jahrhunderten zieht die Universität zahlreiche Gelehrte an, Hölderlin und Uhland verbrachten hier viele Jahre ihres Lebens. Heute sorgen weit über 25.000 Studierende an den in der gesamten Stadt verteilten Instituten für den ganz besonderen Charakter von Tübingen.

Wissenschaft und Forschung werden hier seit jeher groß geschrieben. Der Schwerpunkt Mikrobiologie und Infektionsforschung der Universität Tübingen vereint das gebündelte Wissen medizinischer, pharmazeutischer und biologischer Bereiche und erzielt dadurch bedeutsame Erkenntnisse. Die Universität hat sich weltweit hohes Ansehen in diesem Bereich erarbeitet. Deshalb freue ich mich besonders, dass die diesjährige Tagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie in Tübingen stattfindet. Forschungsergebnisse der Mikrobiologie kommen unserer Gesellschaft durch medizinische Fortschritte zugute und sind Grundlage für zukunftsweisende Maßnahmen.

Für die Tagung wünsche ich Ihnen gutes Gelingen, fruchtbare Diskussionen und Ergebnisse, um die Forschung der Mikrobiologie weiter voranzubringen. Vielleicht finden Sie am Rande der Veranstaltungen die Gelegenheit, Tübingen mit seiner schönen Altstadt und der malerischen Neckarfront kennenzulernen. In der Stadt der kurzen Wege lassen sich die vielen Sehenswürdigkeiten leicht zu Fuß erkunden.

Ich wünsche Ihnen einen angenehmen Aufenthalt in der Universitätsstadt Tübingen!

Ihr

Boris Palmer
Oberbürgermeister der Universitätsstadt Tübingen

Greetings of the President of the VAAM to the Annual Conference 2012

Dear Colleagues and Friends,

The highlight of each year for VAAM is its Annual Conference. The strength and success of our organization is built to a large extent on a long series of very successful conferences, where students and professionals from academia, industry and governmental organizations get together for an intense exchange of scientific information. One very remarkable characteristic of our Annual Conference is the active participation of many students and young scientists, either giving presentations or displaying posters. Offering this forum to them is an important aspect, one that supports scientific development and the integration into the VAAM scientific community.

The attractiveness of our Annual Conference is reflected in the high number of participants. We had about 1500 attendees in Karlsruhe last year and in Tübingen, we expect far more than 1200 participants. The organizers of our conference have put together a very interesting program; the topics are:

Bacterial differentiation, cell envelope, human microbiota, metabolic regulation and signaling, microbial pathogenicity, microbial survival strategies, secondary metabolites and soil microbiology.

In addition, there will be symposia held by our "Fachgruppen" and we will organize a session concerning microbiology in industrial applications, along with speakers from industry. Tübingen will also mark the fifth anniversary of our VAAM Research Award, and I'm very proud that we will be bestowing this year's award to an outstanding female researcher in the field of microbiology.

I'm also very glad to announce that Prof. Gerhard Gottschalk will become an honorary member of VAAM. The award ceremony will be held on March 20th, 2012 during the general meeting.

Prof. Gottschalk is an internationally renowned and distinguished researcher and has been a member of VAAM since our organization was founded. It is a great honor for VAAM to have Prof. Gottschalk as its honorary member.

Last but not least, on behalf of VAAM I want to thank Wolfgang Wohlleben, the members of the Organization Committee, Conventus and Katrin Muth for their great efforts and commitment in organizing the Annual Conference in Tübingen. I would also like to thank all scientists for their contributions. I'm convinced that we will have an excellent conference and enjoyable discussions.

I would like to encourage you to join us in Tübingen for this outstanding scientific event.

See you in Tübingen,

Gerhard Schmid
President of the VAAM

Welcome Address of the Organizing Committee

■ It is our pleasure to welcome you in Tübingen for the 2012 Annual Conference of the Association for General and Applied Microbiology (VAAM). The Eberhard Karls University of Tübingen, which hosts this conference, is one of Germany's oldest universities, founded in 1477. Microbiological research is strongly represented in this university, as well as in Tübingen's renowned Max Planck Institute, in research institutes operated in public/private partnership and in small biotech companies around Tübingen. The importance placed on microbiology is illustrated e.g. by the DFG-sponsored collaborative research center 766 ("The Bacterial Cell Envelope"), by the participation of Tübingen in the German Center for Infectious Disease Research (DZIF) and by the establishment of the Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT) at the university.

The program of the VAAM conference in Tübingen comprises key topics like bacterial differentiation, cell envelope, metabolic regulation and signaling, microbial pathogenicity, microbial survival strategies, secondary metabolites and soil microbiology, as well as specialized minisymposia. 16 plenary lectures, 168 short lectures and about 600 posters will reflect the exciting recent advances of microbiological research in the genomic and post-genomic era. All lectures will take place at the University Campus "Morgenstelle" (Hörsaalzentrum Morgenstelle), allowing close interactions between the participants as well as easy access to the scientific posters and to the exhibitions. The neighboring Botanical Garden, with its impressive greenhouses and its beautiful view to the hills of the Swabian Alb, invites for a visit between the lectures, while the university cafeteria offers food and refreshments. The center of Tübingen, with its wood-frame houses, its narrow old streets and its magnificent facades to the Neckar river, belongs to the most beautiful late medieval towns in Germany. In the evening, lively student pubs, traditional wine bars, beer halls and restaurants offer entertainment for many tastes.

Do not miss a visit to the castle, which dominates the old town of Tübingen. It belongs to the University, and its museum displays the most ancient pieces of art of mankind: wonderful small ivory sculptures of animals, unearthed in the vicinity of Tübingen and dated to the early stone age (35000 – 40000 B.C.). In the courtyard at the castle, look for the sign which marks the laboratory of Hoppe-Seyler, where DNA ("Nuclein") was discovered by Friedrich Miescher in 1869. Furthermore, consider a visit to the enchanting old monastery of Bebenhausen, just a few kilometers from the conference venue.

We sincerely hope that you will enjoy your stay in Tübingen, that you will find the scientific program of the conference stimulating, and that this meeting will contribute to the advancement and recognition of microbiological sciences! ■

***Wolfgang Wohlleben**, Ingo Autenrieth, Volkmar Braun, Karl Forchhammer, Friedrich Götz, Rüdiger Hampp, Lutz Heide, Andreas Kappler, Andrei Lupas, Andreas Peschel*

General Information

2012 Annual Conference of the VAAM

Venue

Eberhard Karls Universität Tübingen
Hörsaalzentrum Morgenstelle
Auf der Morgenstelle 16
72076 Tübingen, Germany

Address for correspondence

Conventus Congressmanagement & Marketing GmbH
Isabelle Lärz
Carl-Pulfrich-Strasse 1
07745 Jena, Germany
Tel.: +49 (0)3641 311 63 20
Fax: +49 (0)3641 311 62 43
www.vaam-kongress.de

Opening hours

Sunday	18.03.	13:30 – 18:30
Monday	19.03.	07:30 – 19:30
Tuesday	20.03.	07:30 – 19:30
Wednesday	21.03.	09:00 – 13:30

Travelling to Tübingen

By train

The main train station in Tübingen is located south of the city centre and approximately 4km from the conference venue.

From there you can take the bus.

By public transport in Tübingen

Conference tickets can be ordered at www.vaam-congress.de until 9 March 2012. Purchase may also be available on-site but not guaranteed.

There are three bus stations near the Morgenstelle.

- Botanischer Garten – with the routes 5, 13, or 17 towards Waldhäuser Ost, Wanne Kunsthalle or Kliniken respectively
- BG Unfallklinik – with the routes 5, 13, or 17
- Auf der Morgenstelle – with route 18 (towards Hagelloch)

For detailed directions on how to get to the Morgenstelle (or anywhere in the city), call +49 (0)7471 93 01 96 96 or visit www.svtue.de.

From south via motor-way A81:

Leave the motor-way via exit #28 Herrenberg, then turn right to Tübingen on federal road B28. Continue along B28 for about 14 km until reaching Tübingen.

Address for navigation systems: Auf der Morgenstelle 16, Tübingen.

From north via motor-way A8 or Stuttgart Airport:

Leave the motor-way via exit #53 and continue onto federal road B27.

Address for navigation systems: Auf der Morgenstelle 16, Tübingen.

Car park

The lecture hall building of Morgenstelle offers free for conference attenders at the "Südparkplatz". Near the Morgenstelle you can also find the carpark Ebenhalde.

Car park Ebenhalde fees:

0.50 EUR	per hour or part thereof
4.00 EUR	daily maximum
1.00 EUR	evenings (19.00–8.00 following day)
1.00 EUR	Sundays and holidays (8.00-8.00 following day)

Address for navigation systems: Schnarrenbergstraße 158, Tübingen.

Hotel reservation

Unfortunately, the room allocation in Tübingen has been exhausted as far as possible. However, rooms can still be arranged on an individual basis through the Tourist and Ticket Center Tübingen.

Neither the Tourist and Ticket Center nor Conventus GmbH can guarantee accommodation directly in Tübingen. Please organize your accommodation as soon as possible. We also suggest making use of private lodgings and guesthouses in and around Tübingen or hotels in Reutlingen and Rottenburg with easy access to Tübingen.

Contact

Bürger und Verkehrsverein
Tourist and Information Center
Marco Schubert
An der Neckarbrücke 1
72072 Tübingen
Tel. +49 (0)7071 91 36 14
marco.schubert@tourist-ticket-center.de
www.tuebingen-info.de

Registration and conference fees

Online registration is possible till 15 March 2012 on the conference homepage at www.vaam-kongress.de. Registration after this date is possible on-site only. Beside cash payments we also accept credit cards at the conference reception desk (Master/ Euro, VISA, American Express and JBC) as well as EC-Cards.

Should you transfer your invoice amount within 10 days of the start of the event, please present your transfer remittance slip at the Check-In desk as proof of payment.

Mixer

The Mixer will take place on Tuesday, 20 March 2012 at 19:30 at the Mensa of the Eberhard Karls Universität near the Morgenstelle. Accompanying persons may purchase a ticket for the mixer at the conference reception desk.

Posters

Posters should be displayed during the whole conference and are divided according number into two poster sessions. All odd poster numbers are in the poster session on Monday, 19 March 2012, 15:15-17:30. Even poster numbers are in the poster session on Tuesday, 20 March 2012, 15:30-17:30.

Posters are to be presented in English and in the format DIN A0 (84.1 cm x 118.9 cm) and no lamination. Authors are asked to attach to the posters the time when they will be available for discussion. The posters will have to be fixed by pins. Materials will be provided.

The posters may be attached from 14:00 on Sunday, 18.03.2012 and should be removed before 12:00 on Wednesday, 21.03.2012.

Presentation of the Honorary Award, PhD Awards, and Poster Prizes

The presentation of the Honorary Award will take place on 19.03.2012 at 11:00.

The presentation of the PhD thesis prizes will take place on 20.03.2012 at 17:30.

The presentation of the poster prizes will take place on 21.03.2012 at 13:00.

All awards will be presented in the lecture hall N6.

Short lectures

The length of short lectures has been fixed to a max. of 10 minutes plus 5 minutes for discussion (some variation may apply). Due to the fact that there will be up to 7 parallel sessions please adhere to the total time allotted to you. Each lecture will have a countdown. At the end of your speaking time the screen will turn black.

Short lectures are to be held in English. Data projectors are available in each of the lecture halls. In each lecture hall there will be an assistant for technical support. We ask all lecturers to make use of the computer facilities located at our presentation submission to check their presentations in advance.

General Tips for Authors and Presenters

Presentation Submission desk

Follow the signs or ask at the Check-In desk how to find the presentation submission.

Time Allotment

To ensure smooth running of the entire programme, all speakers are advised to adhere to their allocated speaking time. The chair persons of the sessions are urged to cancel discussions in delay. Contact your chair person before your session begins and advise of any changes or special wishes.

Presentation Form and Submission of Presentation

Please submit your presentation at least 120 minutes before your lecture will start. You are asked to clearly label your CD/memory stick and the file with your short lecture code number and the name of the person giving the talk. All presentations will be loaded onto our computers and will be deleted after the talks.

PDF and PowerPoint presentations are permitted. Open Office formats may also be used. Required technical equipment will be available at the congress.

The use of Macintosh or Open Office formats as well as the use of a personal laptop for a presentation is not planned, but possible. If necessary, please contact us by 15 February 2012 at vaam-kongress@conventus.de.

For video and audio files please submit AVI, WMV and MPG files only as a separate file.

Please make sure that any required CODEC files for any videos are also submitted.

Your presentation and any additional files should be handed over at least two hours before your presentation time.

Please note: If you use a USB stick to save your files, do not protect it with software.

Registration fees (all days)

VAAM-Members	
Regular	210 €
Student*	85 €
Industry Representatives**	300 €
Retiree	100 €
Non-members	
Regular	280 €
Student*	110 €
Industry Representatives**	145 €
Retiree	370 €
Fee for day tickets (Monday, Tuesday, Wednesday)	**100** €

* Please provide confirmation and quote VAAM 2012 as the reference.

** This fee is not for industry representatives taking part in the industrial exhibition. Special rates will be provided for booth personnel.

Social programme

Welcome reception*** (18.03.2012)	included
Mixer*** (20.03.2012)	included

*** Registration required.

Sponsoren und Aussteller der Jahrestagung der VAAM 2012/ Sponsors and Exhibitors of the Annual Meeting of the VAAM 2012

(Stand: 17.02.2012/as of: 17.02.2012)

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Boehringer Ingelheim Pharma KG (Biberach a.d. Riss)
Caister Academic Press Ltd (Norwich/UK)
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Wacker Chemie AG (München)

Sponsor Posterpreise/Sponsor posterprizes

MorphoSys AG (Planegg-Martinsried)

Förderungen/Funding

Federation of European Microbiological Societies (FEMS)
www.fems-microbiology.org

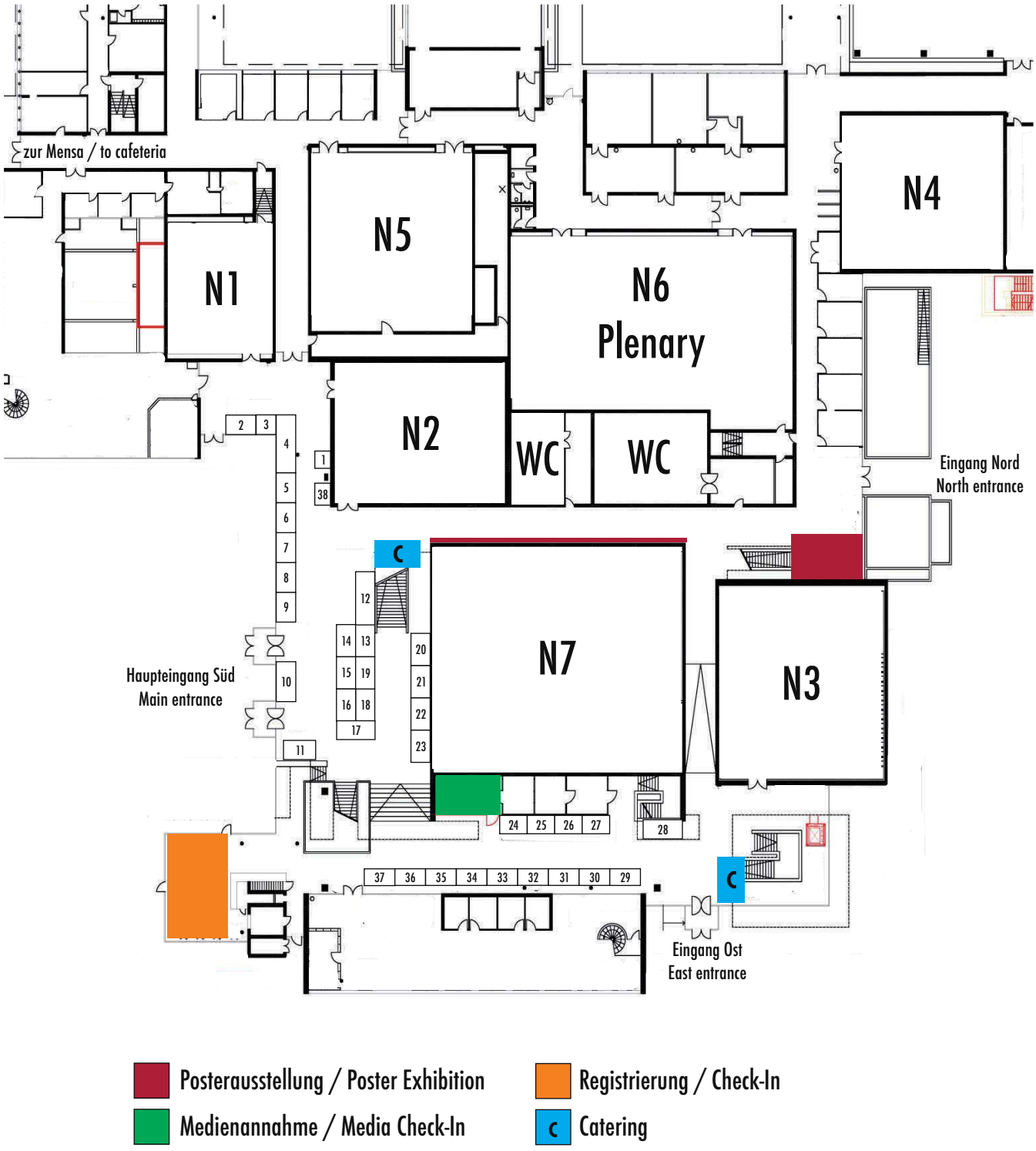
Universitätsbund Tübingen
Vereinigung der Freunde der Eberhard Karls Universität e.V.
http://homepages.uni-tuebingen.de/Unibund/

Aussteller/Exhibitors

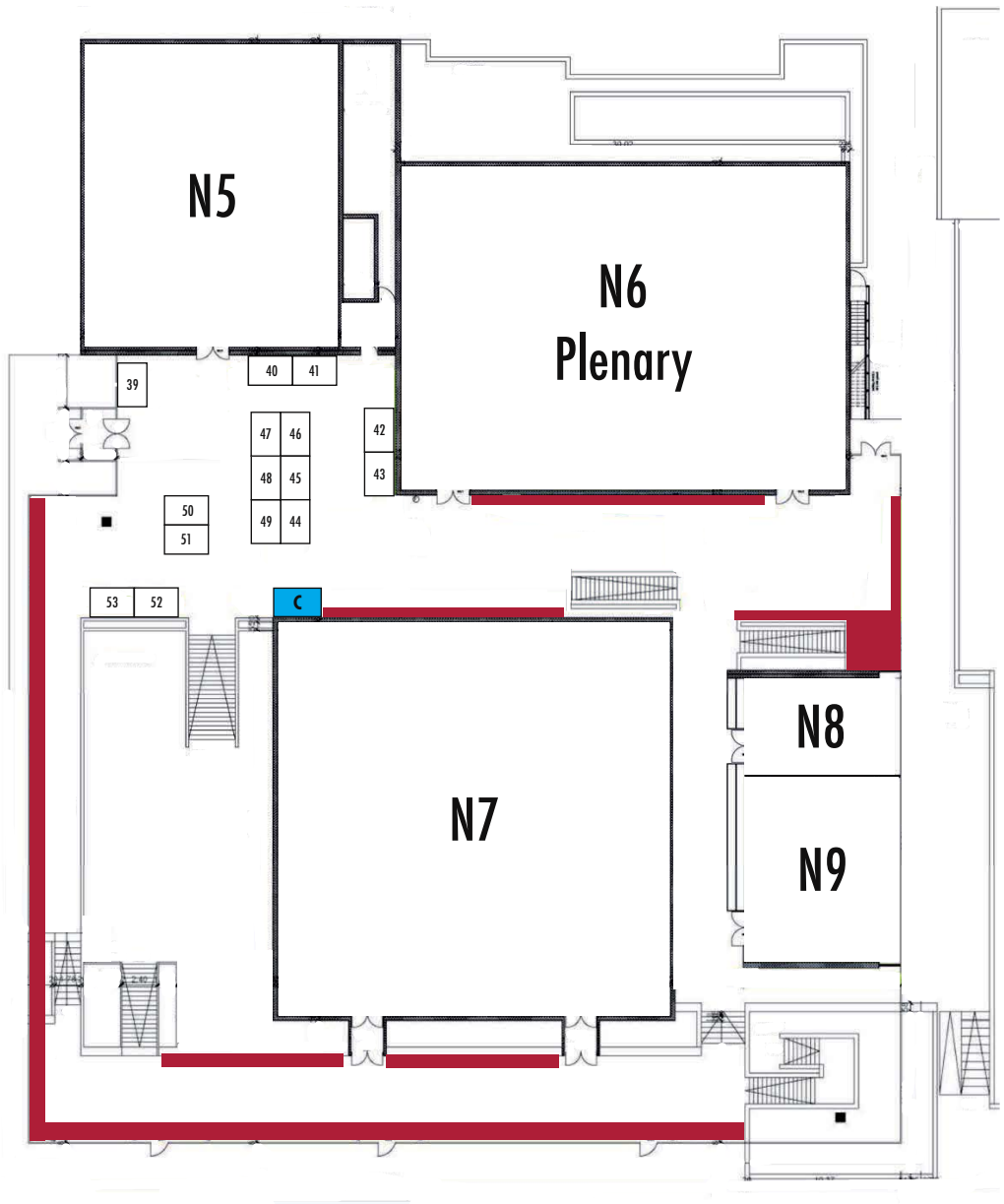
Alphabetische Reihenfolge/Alphabetical order

Numerische Reihenfolge/Numerical order

Morgenstelle Erdgeschoss / Morgenstelle Ground Floor



Morgenstelle Obergeschoss / Morgenstelle First Floor



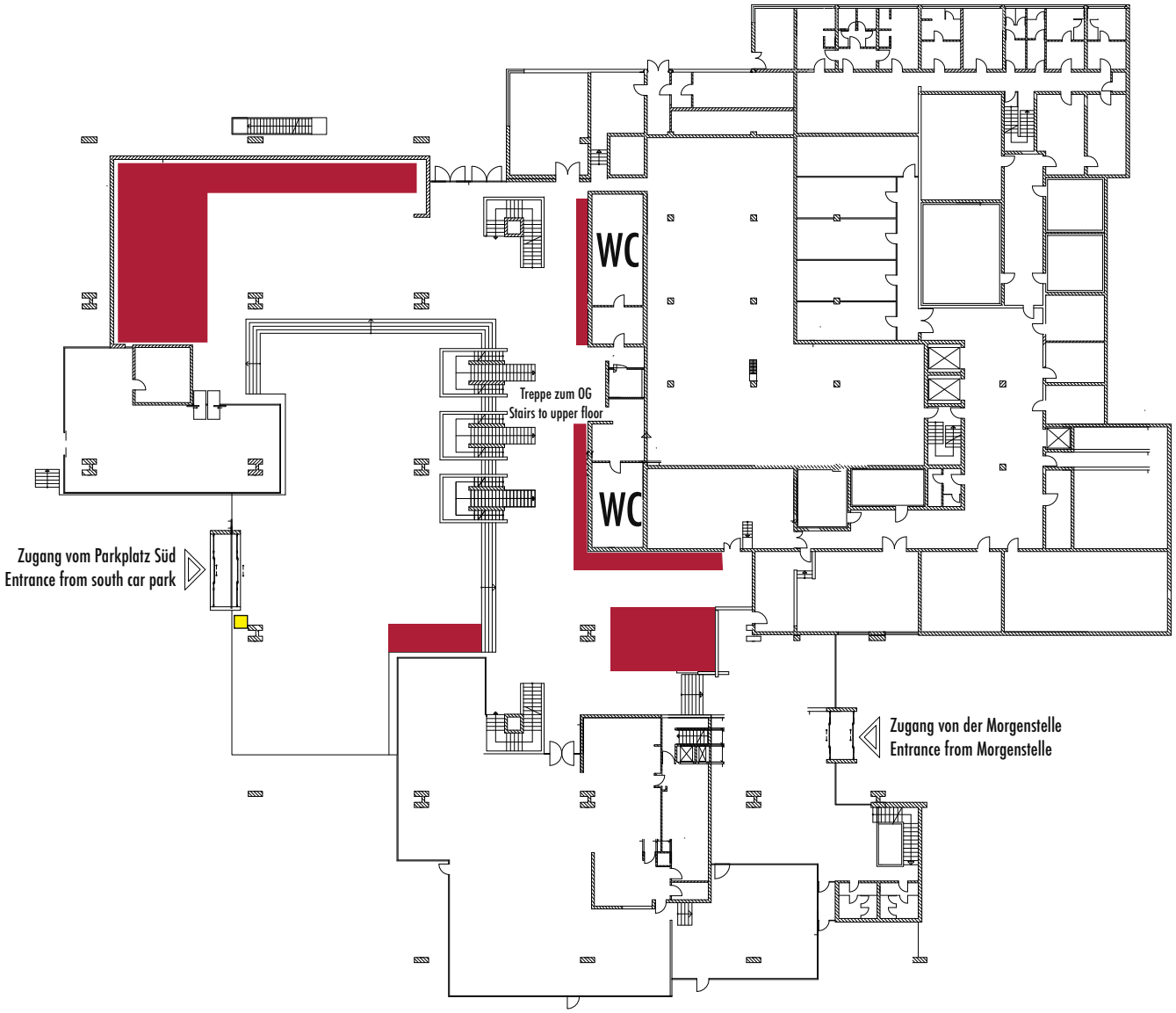
Posteraussstellung / Poster Exhibition

Registrierung / Check-In

Medienannahme / Media Check-In

C Catering

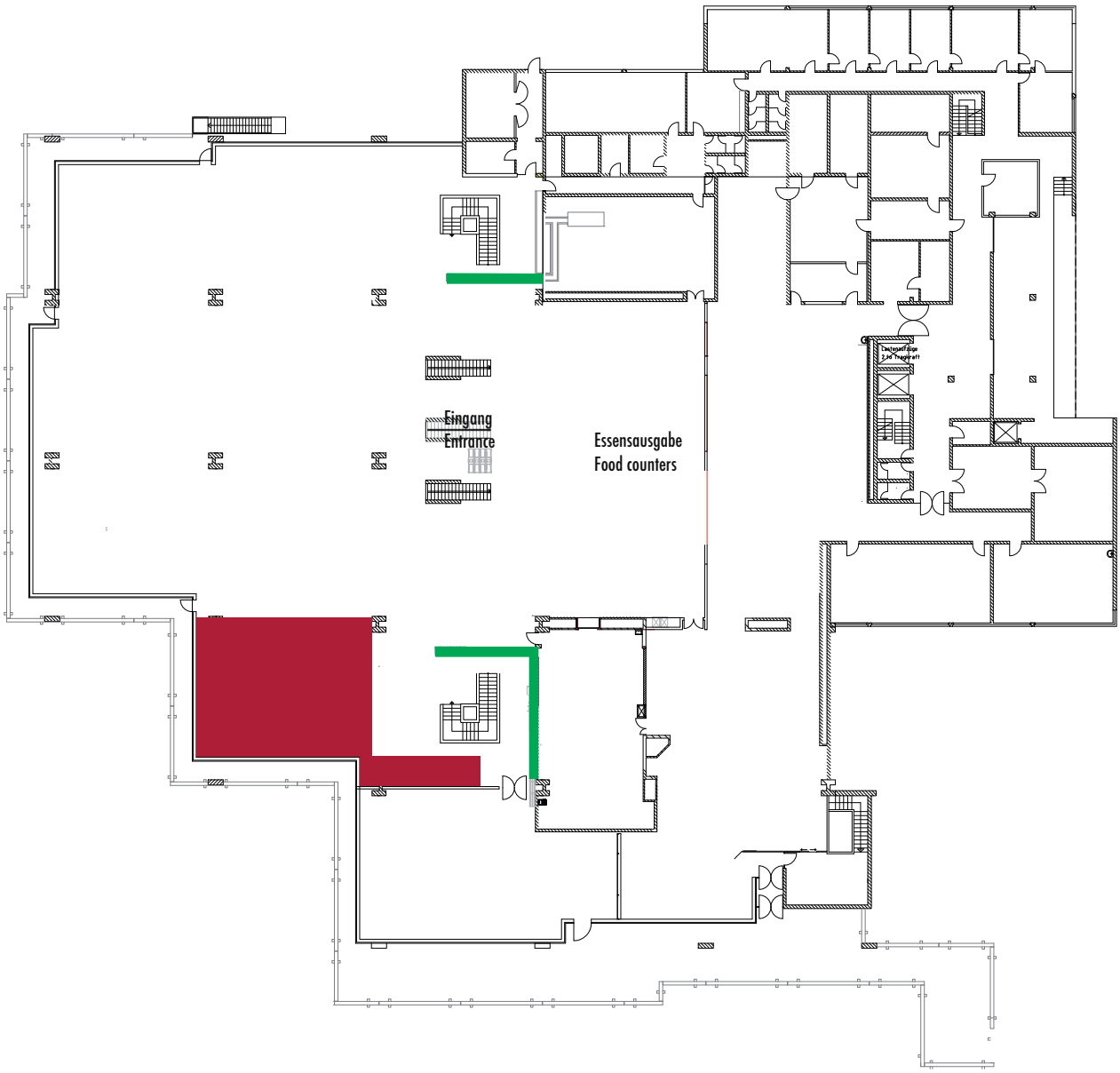
Mensa Eingangshalle / Cafeteria Entrance Hall



Posterausstellung / Poster Exhibition

Geldautomat / Cashpoint

Mensa Eben Speisesaal / Cafeteria Dining Hall



Posterausstellung / Poster Exhitibion

Geschirrrückgabe / Dish Return

Einladung zur Mitgliederversammlung der VAAM

■ Hiermit lade ich alle Mitglieder der VAAM zur Mitgliederversammlung ein. Sie wird am Dienstag, den 20. März 2012, um 17.30 Uhr im Hörsaalkomplex Morgenstelle der Eberhard-Karls-Universität Tübingen (Hörsaal N6) stattfinden.

Vorläufige Tagesordnung:

1. Festlegung der Tagesordnung und Genehmigung der Niederschrift der Mitgliederversammlung vom 5. April 2011 in Karlsruhe (siehe BIOspektrum 4/11, Seiten 447 und 448)
2. Bericht aus dem Vorstand, u.a. Haushalt 2011 und Haushaltsplan 2012, Ort und Zeit der nächsten Jahrestagung, Aktivitäten der Fachgruppen, VBIO, Öffentlichkeitsarbeit
3. Bericht der Kassenprüfer
4. Entlastung des Vorstandes
5. Änderung der Geschäftsordnung (Fachgruppen), s.u.
6. Wahl eines Ehrenmitglieds
7. Verschiedenes

Im Anschluss:
- Verleihung der Ehrenmitgliedschaft an Gerhard Gottschalk
- Verleihung der VAAM-Promotionspreise 2012

Reisekostenzuschüsse für studentische Mitglieder können bei fristgerecht eingegangenen Anträgen und bei Vorliegen der sonstigen Voraussetzungen nur persönlich am **Dienstag, den 20. März 2012 zwischen 14.00 Uhr und 16.00 Uhr** sowie am **Mittwoch, den 21. März 2012, zwischen 10.00 Uhr und 12.00 Uhr** im Tagungsbüro abgeholt werden. ■

Hubert Bahl
Schriftführer

Änderung der VAAM-Geschäftsordnung, Punkt V.

Neuerungen sind farblich hervorgehoben

Einrichtung von Fachgruppen

■ Mitglieder der Gesellschaft mit besonders spezialisiertem fachlichen Interesse können sich innerhalb der Gesellschaft zu Fachgruppen zusammenschließen. Der Antrag auf Einrichtung einer Fachgruppe muss von mindestens 25 ordentlichen Mitgliedern unterstützt werden und ist an den Präsidenten zu richten. **Die Mitgliederversammlung beschließt die Einrichtung von Fachgruppen für vier Jahre mit einfacher Mehrheit. Eine Verlängerung der Dauer für jeweils weitere vier Jahre ist auf begründetem Antrag durch den Vorstand möglich.** Die Fachgruppen können Symposien abhalten und innerhalb von Tagungen der VAAM ihr Spezialgebiet vertreten. Die Vertretung der Fachgruppen nach außen ist Angelegenheit der VAAM bzw. ihres Präsidiums. Den Fachgruppen wird jeweils ein vom Präsidium festgesetzter fester Betrag für ihre Aktivitäten zur Verfügung gestellt, der mit Zustimmung des Schatzmeisters in das jeweils nachfolgende Jahr übertragen werden kann. Die Mitglieder der Fachgruppen wählen auf einer Mitgliederversammlung aus ihrer Mitte – jeweils auf 2 Jahre – einen Sprecher der Gruppe und seinen Stellvertreter, was durch das Präsidium der VAAM zu bestätigen ist. Wiederwahl ist zulässig. Die Sprecher der Fachgruppen berichten über deren Aktivitäten im Vorstand und in der Mitgliederzeitschrift oder im Tagungsband der Jahrestagung der VAAM.

Das zurzeit amtierende Präsidium regelt den Übergang von der bisherigen auf die neue Geschäftsordnung. ■

Karrieresymposium

Vorstellung vielfältiger Berufsbilder in den Biowissenschaften
Anregungen und Tipps zur Karriereplanung

Montag, 19. 03. 2012
15.45 – 17.15 Uhr
Hörsaal N6

Marion Karrasch, Forschungszentrum Jülich
„Karrieremöglichkeiten in der Forschungsförderung“

Meike Kammler, Universität Tübingen
„Perspektiven in der Wirtschaftsförderung – als Netzwerkerin zwischen Wirtschaft, Wissenschaft und Politik“

Oliver Müller, Capgemini Consulting
„Vom Forscher zum Berater – als Naturwissenschaftler in der Strategie- und Managementberatung“

Die Veranstaltung wird unterstützt und gefördert durch das BMBF im Rahmen des ESIT-Projekts „Erfolgreich studieren in Tübingen“.

International Conference on Microbiology

and

Koninklijke Nederlandse Vereniging voor Microbiologie

Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM)

zusammen mit der

Koninklijke Nederlandse Vereniging voor Microbiologie (KNVM)

Annual Conference of the Association for General and Applied Microbiology (VAAM)

in collaboration with the

Royal Netherlands Society for Microbiology (KNVM)

Main Topics:

- Host-Microbe Interaction
- Marine Microbiology
- Physiology and Metabolism
- Omics and Bioinformatics
- Unicellular Eukaryotic Microbiology
- Food and Feed Microbiology
- Environmental Biotechnology
- Single Cell Microbiology

BREMEN 2013

10–13 MARCH 2013

Congress Center Bremen und Messe Bremen

Quelle Skyline: www.fotolia.com • Matthias Enter

Abstract Submission, Registration and Information: www.vaam-kongress.de

Fachgruppe: Archaea

Archaea bilden die dritte Domäne des Lebens und werden häufig mit extrem heißen, sauren oder salzigen Standorten auf Kontinenten oder in der Tiefsee in Verbindung gebracht. Sie sind zwar charakteristische Bewohner dieser Habitate, die methanogenen Archaea kommen aber auch in weniger exotischen Standorten wie dem Pansen von Wiederkäuern vor. Die Bedeutung der Archaea hat in den letzten Jahren stark zugenommen, da über genetische Methoden viele Vertreter dieser Organismengruppe im Boden oder Süßwasser entdeckt wurden, die noch nicht kultiviert wurden, aber am N-Kreislauf stärker beteiligt sind als bislang vermutet. Aufgrund der phylogenetischen Stellung sind sie spannende Forschungsobjekte im Hinblick auf Evolution von Enzymen; auch ihre Anpassung an die extremen Umweltbedingungen ist häufig sehr spezifisch, und die Handhabung dieser Organismen im Labor erfordert spezielle Adaptionen. Neben der Kultivierung ist dies bei allen physiologischen, biochemischen oder molekulargenetischen Untersuchungen der Fall; gängige Gelsysteme funktionieren beispielsweise nicht bei 5 M NaCl oder 90°C.

Die Fachgruppe Archaea schafft ein Forum, in dem neben Präsentation und Diskussion wissenschaftlicher Daten auch Erfahrungen bei der Entwicklung von Untersuchungsmethoden ausgetauscht werden. Mitglieder der Fachgruppe beschäftigen sich mit einem breiten Spektrum zur Ökologie, Physiologie, Molekularbiologie und den Zellstrukturen von Archaea und können immer wieder spannende Erkenntnisse oder neue Entdeckungen präsentieren. Gemeinsamer Treffpunkt ist die jährlich im Herbst von Jörg Soppa organisierte Tagung in Schmitten bei Frankfurt, bei der vor allem Doktoranden ihre Daten zur Diskussion stellen und ein bis zwei internationale Sprecher zum Vortrag eingeladen werden.

Sprecherin: Felicitas Pfeifer, Universität Darmstadt
Email: pfeifer@bio.tu-darmstadt.de

Fachgruppe: Mikrobielle Pathogenität – gemeinsam mit der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM)

Die Fachgruppe bildet ein Forum für die erregerorientierte bakterielle Infektionsforschung in Deutschland und ist gleichermaßen in der VAAM und der DGHM vertreten. Wie schon in den vergangenen Jahren gestalteten wir 2011 die Jahrestagungen der beiden Dachgesellschaften aktiv mit. Bei der VAAM-Tagung in Karlsruhe wurden zwei Sessions zu den Themen ‚Pathogen metabolism and physiology' und ‚Virulence factors' sowie eine gemeinsame Session mit der Fachgruppe Regulation und Signaltransduktion mit dem Thema ‚Second messengers in bacteria' ausgerichtet. Im Rahmen der DGHM-Tagung in Essen fanden Sessions mit Titeln wie ‚Microbial pathogenicity and gastrointestinal infections', ‚Microbial pathogenicity and eukaryotic pathogens', ‚Intracellular pathogens', und ‚Interaction of pathogens with host cells and matrix proteins' statt. Ein gut besuchtes internationales Symposium zum Thema ‚How dead is dead?', organisiert von Ralf Bertram und Friedrich Götz, fand mit Unterstützung der Fachgruppe im Juni in Tübingen statt.

Im Jahr 2012 stehen wichtige Entscheidungen und Aktivitäten an. Anfang des Jahres werden die Mitglieder der Fachgruppe die Sprecher für die VAAM-Fachgruppe neu wählen. Gleiches gilt auch für den Vorstand der DGHM-Fachgruppe, aus dem Volkard Kempf ausscheiden wird. Bei der VAAM-Jahrestagung in Tübingen wird das Thema Mikrobielle Pathogenität eine große Rolle spielen, mit der Chance, Sitzungen zu verschiedenen Themen der Fachgruppe organisieren zu können. Vom 18. bis 20. Juni 2012 wird zum sechsten Mal die traditionell alle zwei Jahre stattfindende Tagung der Fachgruppe in Bad Urach stattfinden. Bei dieser haben vor allem jüngeren Mitgliedern die Gelegenheit, ihre wissenschaftlichen Arbeiten zu präsentieren und sich auszutauschen. In diesem Jahr wird auch die 3. Nationale *Yersinia*-Tagung in Tübingen stattfinden, die Ingo Autenrieth organisiert und die ebenfalls von der Fachgruppe unterstützt wird. Schließlich wird die Fachgruppe auch bei der DGHM-Jahrestagung in Hamburg präsent sein und aktiv mehrere ‚Sessions' organisieren.

Der Vorstand wünscht den Mitgliedern der Fachgruppe ein gesundes und erfolgreiches Jahr 2012.

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Holger Rohde

Fachgruppe: Fungal Biology and Biotechnology/Experimentelle Mykologie

■ Ziel unserer Fachgruppe ist es, Doktoranden, Post-Docs und Habilitanden, also unserem wissenschaftlichen Nachwuchs zweimal im Jahr ein Forum zur Diskussion eigener Ergebnisse zu bieten.

Im Jahr 2011 konnten wir dieses Ziel erreichen. Wir danken Prof. Dr. Michael Bölker für die Organisation der Traditions-Tagung „Molekularbiologie der Pilze“ im Herbst in Marburg und PD Dr. Matthias Brock sowie Prof. Dr. Stefanie Pöggeler für das Minisymposium „Fungal Development and Pathogenicity Mechanisms“ im Rahmen der Frühjahrstagung in Karlsruhe.

Zur Frühjahrstagung 2012 in Tübingen wird das Fachgruppen-Minisymposium „Fungi go omics – Fungal systems biology approaches in the postgenomic era“ stattfinden. Die Veranstaltung ist für Montag, den 19. März vorgesehen. PD Dr. Sven Krappmann (Würzburg) wird sie zusammen mit Prof. Dr. Vera Meyer (Berlin) leiten. Gastsprecher ist Dr. Cees Sagt (Delft, NL), der über „Applied genomics in an industrial setting“ sprechen wird. Anschließend werden sechs Kurzvorträge von Doktoranden stattfinden, die anhand der eingereichten Abstracts ausgewählt wurden.

Im Anschluss an das Minisymposium wird die Fachgruppensitzung stattfinden. Dort sollen Ideen für Veranstaltungen in Jahr 2013 diskutiert werden. Interesse hat bereits Prof. Dr. Dirk Hoffmeister (Jena) angemeldet, der mit Basidiomyceten arbeitet.

Ein Tagungshöhepunkt für alle Pilz-Begeisterten verspricht die von Prof. Dr. Regine Kahmann nach Marburg geholte „11th European Conference on Fungal Genetics – ECFG 11“ zu werden. Die Fachgruppe unterstützt die Veranstaltung, die vom 30.3. bis 2.4.2012 stattfinden wird, denn sie bietet unseren Doktoranden die Gelegenheit, unmittelbar mit international angesehenen Wissenschaftlern in Kontakt zu kommen. ■

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Fachgruppe: Struktur und Mikroskopie

■ Unsere Fachgruppe richtet auf den VAAM-Tagungen jedes zweite Jahr Minisymposien aus, die sich bisher im Wechsel mit mikroskopischen und mikrobiologisch-strukturellen Themen beschäftigt haben. Die mikroskopischen Techniken vollzogen in den letzten fünfzehn Jahren eine spannende Entwicklung, die auch die mikrobiologische Strukturforschung berührt und beeinflusst hat. Wenn zurückliegende Symposien deshalb Fluoreszenztechniken wie die Konfokale Laserscanning-Mikroskopie (CLSM),*Total Internal Reflection Fluorescence Microscopy* (TIRF) und die *Stimulated Emission Depletion Microscopy* (STED), weiterhin die Rastersondenmikroskopien und Kryo-Elektronentomographie einmal oder gar mehrmals zum Thema hatten, lag es an ihrem Potenzial für die Erforschung zellulärer Strukturen und Eigenschaften. Die Methoden sind mittlerweile in der Mikrobiologie angekommen und werden genutzt, um bisher schwer erfassbare oder unbekannte (sub)zelluläre Zustände und Änderungen sichtbar zu machen. So konnten wir uns in Fachgruppen-Symposien über das bakterielle Cytoskelett, das Phänomen des *Macromolecular Crowding* und anlässlich der Frühjahrstagung 2011 in Karlsruhe über die Struktur des Cytoplasmas informieren lassen (Berichte finden Sie im BIOspektrum). Diese Themen sind Aspekte eines Fachgebiets, das sich zusammen mit den neuen mikroskopischen Methoden entwickelt, zunehmend an Bedeutung gewonnen und sich als *Mikrobielle Zellbiologie* etabliert hat.

Es liegt nahe, und es lohnt sich deshalb darüber nachzudenken, den wissenschaftlichen Schwerpunkt zukünftiger Aktivitäten unserer Fachgruppe mehr in einen integrativen biologischen Rahmen der Strukturforschung zu legen und damit der mikrobiellen Zellbiologie ein Forum zu bieten, das sie in unserer Fachgesellschaft noch nicht explizit vorfindet.

Sie, alle Fachgruppen-Mitglieder und alle Interessentinnen und Interessenten, sind deshalb herzlich eingeladen, die thematische Ausrichtung unserer Fachgruppe auf der Mitgliederversammlung anlässlich der VAAM-Tagung in Tübingen zu diskutieren und mitzugestalten.

Unsere FG-Sitzung findet am Montag, 19. März 2012, von 17.00 bis 17.30 Uhr im Hörsaal N8 statt. Die Themen: *(1.) Ausrichtung der Fachgruppe, (2.) Zukünftige Symposien, (3.) Ergebnis der Sprecherwahl.* Ich freue mich auf Ihr Kommen und auf Ihre Anregungen! ■

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Fachgruppe: Biologie bakterieller Naturstoffproduzenten

Die Fachgruppe „Biologie bakterieller Naturstoffproduzenten“ beschäftigt sich mit Themen der Naturstoffbiosynthese, vor allem in Actinomyceten, Myxobakterien und Bacilli. Das Interessensgebiet der Fachgruppe umfasst sowohl die Grundlagen als auch die angewandte Forschung. Die insgesamt rund 200 Mitglieder kommen aus der Hochschule und aus der Industrie.

Neben Kolleginnen und Kollegen aus der Mikrobiologie sind auch vermehrt mikrobiologisch orientierte Chemiker, Biochemiker und Pharmazeuten Mitglieder unserer Fachgruppe.

Einmal im Jahr organisiert die Fachgruppe einen Workshop, der vor allem jungen Wissenschaftlern ein Forum bietet, ihre Ergebnisse einem größeren Kreis interessierter Kollegen vorzustellen und zu diskutieren. Der Workshop 2011 wurde im September an der Universität Bonn von Prof. Gabriele König und Prof. Jörn Piel organisiert (s. Bericht im BIOspektrum 07/2011, S. 799). Neben Vorträgen zur „Biochemie und Genetik der Sekundärmetabolitproduktion“ gab es vor allem Berichte zu neuartigen Methoden der Bioinformatik und Massenspektroskopie, mit denen neue Wirkstoffe identifiziert werden können. Aber auch Beiträge zu verschiedenen Aspekten der Streptomycetenbiologie, von der Rolle kleiner RNAs in diesen Bakterien bis zum Gentransfer nahmen einen breiten Raum ein.

Die Fachgruppe beteiligte sich darüber hinaus an der Organisation internationaler Tagungen, wie dem „16th International Symposium on the Biology of Actinomycetes“ (ISBA) im Dezember 2011 in Mexiko.

Im nächsten Jahr wird es neben dem traditionellen Workshop (voraussichtlich im September in Frankfurt) eine Summerschool geben. Organisiert wird diese Tagung von Prof. Hildgund Schrempf in Münster vom 25. bis 28. Oktober 2012 zum Thema „Actinobacteria within soils: capacities for mutualism, symbiosis and pathogenesis“. Außerdem ist zusammen mit dem Arbeitsausschuss „Niedermolekulare Naturstoffe“ der DECHEMA eine gemeinsame internationale Tagung (European Conference on Natural Products: Research and Applications) vom 22. bis 25. September 2013 in Frankfurt geplant.

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Fachgruppe: Symbiotische Interaktionen

Die Symbioseforschung kann in Deutschland auf eine lange wissenschaftliche Tradition zurückblicken. Die 2009 gegründete Fachgruppe „Symbiotische Interaktionen“ ist jedoch die jüngste Gruppe der VAAM und umfasst rund 90 Mitglieder. Das Ziel dieser Fachgruppe ist es, die verschiedenen Themen und Modelle der Symbioseforschung in Deutschland zu bündeln und auf internationaler Ebene sichtbar zu machen, den Informationsaustausch zwischen Universität und Industrie zu fördern sowie junge Wissenschaftler für die Symbioseforschung zu begeistern. Das inhaltliche Themenspektrum umfasst verschiedene Modelle der Interaktion von Bakterien mit ihren Wirten, die aus den Bereichen Pflanze, Tier, Mensch rekrutiert werden. Wer neugierig geworden ist, erhält weitere Informationen zu inhaltlichen Themen und methodischen Ansätzen auf der Homepage (http://www.helmholtz-muenchen.de/en/symbiotic-interactions/).

Der wissenschaftliche Austausch in der Fachgruppe findet vorwiegend auf den Mini-Symposien bei den VAAM-Jahrestagungen statt. So wird auf der Jahrestagung 2012 in Tübingen ein Minisymposium zum Thema „Symbiotic Interactions“ organisiert. Ein besonderes Highlight setzt Gastsprecherin Dr. Virginia Weis, Oregon, USA, die ihre aktuellen Forschungstätigkeiten im Bereich der Symbiose von Korallen und Anemonen mit photosynthetischen Algen (Dinoflagellaten) darstellen wird. Im Zuge der globalen Erderwärmung kommt dem Ausbleichen von Korallen durch Verlust ihrer Symbionten („coral bleaching“) eine besondere Bedeutung zu. Eine halbe Stunde vor Beginn des Minisymposiums werden die Mitglieder der Fachgruppe sowie zukünftige Interessenten zu einer Mitgliederversammlung eingeladen, die im gleichen Tagungsraum wie das Minisymposium stattfinden wird. Neben der Diskussion inhaltlicher und gestalterischer Themen zur Fachgruppe steht turnusgemäß die Wahl der Sprecher an.

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Fachgruppe: Qualitätssicherung und Diagnostik

■ Was haben Lebensmittel, Wandfarbe, Arzneimittel und Chemikalien gemeinsam? In ihnen können Mikroorganismen vorkommen – erwünscht oder notwendig, aber auch als unerwünschte Kontamination. Und wenn sie absichtlich darin vorkommen, sind es auch die richtigen? Stimmt ihre Anzahl? Falls sie nicht erwünscht sind, wie wird man sie wieder los?

Die Aufgabe der Mitglieder der Fachgruppe Qualitätssicherung und Diagnostik ist es, diesen Fragen nachzugehen. Unsere Mitglieder etablieren Methoden zur Detektion und Identifizierung oder verbessern diese, um relevante Bakterien nachzuweisen und ihre Anzahl zu bestimmen. Dieses Wissen ermöglicht es, Maßnahmen für eine sichere Herstellung auszuarbeiten und unerwünschte Mikroorganismen einzudämmen.

Die Fachgruppe versteht sich als ein Forum für Mikrobiologen, die vor allem in Industrieunternehmen in der Forschung & Entwicklung, der Qualitätskontrolle oder der Marktversorgung tätig sind oder in diesem Berufsfeld eine Aufgabe nach dem Studium suchen. Wir treffen uns mindestens einmal jährlich an wechselnden Orten in Deutschland zu Vortrags- und Diskussionsveranstaltungen.

Bei unserem Jahrestreffen am 30. September 2011 bei Provadis im Industriepark Höchst in Frankfurt/Main kamen rund 40 aktive Mitglieder der FG zusammen. Hier wurden beispielsweise schnelle Methoden der Mikrobiologie, die „Rapid Microbiological Methods", vorgestellt und diskutiert. Diese ermöglichen eine schnelle molekularbiologische Artbestimmung von Bakterien ohne zeitraubende Kultivierungsschritte. In naher Zukunft kann eine Bestimmung von Bakterien statt mehrerer Tage durch Koch'sche Verfahren innerhalb eines Tages oder sogar weniger Stunden möglich werden. In Folge erlaubt dies eine schnellere Freigabe von Wirkstoffen und eine zeitnahe Kontrolle von Produktionsprozessen. Die Forschung und Entwicklung der Mikrobiologie wird weiter nach neuen und besseren Methoden suchen, um in das Reich des kleinsten Lebens vorzudringen. Weitere Vortragsthemen waren die EHEC-Epidemie im Frühjahr 2011, die Bewertung neuer Richtlinien für die Verwendung mikrobiologischer Nährmedien, unterschiedliche Identifizierungsmethoden sowie die Zuverlässigkeit von Datenbanken, auf Basis derer z. B. molekularbiologisch erzielte Identifizierungsergebnisse interpretiert werden. Eine Führung durch das berühmte Industriedenkmal Peter-Behrens-Bau, das ehemalige Verwaltungsgebäude der Hoechst AG, lockerte den Tag auf.

Das nächste Treffen wird begleitend zur VAAM-Frühjahrstagung in Tübingen (20.03.2012) stattfinden. Dort werden wir anhand fachlicher Präsentationen durch Sprecher aus verschiedenen Karrierestufen das Berufsbild des „Industrie-Mikrobiologen" erhellen und wollen in einer anschließenden Diskussion jungen Mikrobiologen Berufsperspektiven im Bereich Qualität und Diagnostik aufzeigen. Auch unser regelmäßiges Jahrestreffen im Herbst 2012 ist in Planung, es wird am Freitag 28. September 2012 in Köln in Anschluss an eine Pharmakonferenz (www.aseptikon.de) stattfinden. Wir werden hierbei auch von thematischen Schnittmengen der Sprecher und Aussteller profitieren können.

Die Fachgruppe wird den Austausch mit weiteren Gruppen und Organisationen auf ihrem Gebiet verstärken und für ihre Mitglieder über Internet-basierende Plattformen den Austausch zwischen den Treffen ermöglichen. So ist in XING bereits eine sich stets um weitere Interessenten erweiternde Netzwerkmöglichkeit etabliert (https://www.xing.com/net/pricdf8f4x/mikrobiologie). Neben der Fachgruppe ist hier auch die offizielle Präsentation der VAAM angesiedelt. Dadurch kann ein Austausch über mikrobiologische Fachthemen unserer Fachgruppen –und vielleicht bald weiterer Fachgruppen– sowie der VAAM im Gesamten innerhalb von Fachkreisen erfolgen. ■

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Fachgruppe: Umweltmikrobiologie

■ Nachdem die Fachgruppe 2011 das „International Symposium on Subsurface Microbiology" mit geplant und durchgeführt hat, wird Barbara Morasch, Universität Tübingen für diese VAAM-Jahrestagung ein Minisymposium mit dem Thema „What makes carbon sources difficult for microbes to degrade?" organisieren. Eingeladene Sprecher sind Prof. Dr. Friedrich Widdel, Max-Planck-Institut für Marine Mikrobiologie, Bremen, mit dem Vortragstitel: „Degradation of organic carbon by microorganisms – do we know the 'rules' and limits?" sowie Dr. Frederik Hammes, EAWAG Dübendorf, CH zu dem Thema: „Characterising oligotrophic bacterial growth with flow cytometry". Die weiteren Vorträge werden aus den Abstracts ausgewählt.

Einladung zur Mitgliederversammlung: Im Rahmen des Minisymposiums wird die Fachgruppe auch ihre Mitgliederversammlung abhalten. Ich würde mich freuen, wenn es Interessenten gäbe, die gerne einen Workshop mit Unterstützung der Fachgruppe organisieren möchten. Vorschläge können entweder per Mail an mich geschickt werden (rainer.meckenstock@helmholtz-muenchen.de) oder in der Fachgruppensitzung diskutiert werden. ■

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Fachgruppe: Identifizierung und Systematik

■ A session entitled “From Genes to Cells” was organised at the ICSEB/GfBS conference BioSystematics 2011, 21. bis 27.02.2011, Berlin. Funds were initially made available for the travel of invited speakers in the session to attend the meeting from the VAAM Fachgruppe “Identifizierung und Systematik”, however, the meeting was financially successful and these funds were returned to the VAAM. The topics covered in the session were:

1. Microbial Genomics.
2. Molecular control of bacterial growth and form.
3. Physiological and biochemical diversity of prokaryotes.
4. The chemical diversity of prokaryotes.
5. Standards in Genomic Sciences: A standards compliant open-access journal for the ‘omics community.
6. Exploring niche specialisation in marine microbes by context enabled comparative genomics.

The session held at the VAAM-Jahrestagung, 3. bis 6.04.2011 in Karlsruhe centred on various aspects of microbial lipids, which are traditionally only poorly covered in microbiological text books. The session was well attended and covered the topics of:

1) Lipids – The fourth cornerstone in biological chemistry
2) Biosynthesis and remodelling of bacterial membrane lipids
3) Regulation of membrane homeostasis in *Pseudomonas aeruginosa*
4) Fatty acid synthesis in fungal type I protein complexes
5) Structural analysis of the polar lipids of *Sphingobacterium spiritivorum* and *Pedobacter heparinus*

There was input into another session at the VAAM-Jahrestagung, 3. bis 6.04.2011 on “Science and Infrastructure” dealing with the topic of the reliability of published data and the significance of both the use of authentic strains as well as the ability to verify data published/deposited in databases based on the original biological material used. This message was also conveyed to a meeting of the Society for General Microbiology at its Spring meeting in Harrogate, 11. bis 14.04. 2011, the 4th FEMS Congress of European Microbiologists in Geneva, Switzerland, 26. bis 30.06.2011 and also the meeting of the VAAM Fachgruppe “Qualitätssicherung und Diagnostik” 30.09.2011 in Frankfurt-Höchst.

Concern has been expressed in the Ausschuss für Biologische Arbeitsstoffe – ABAS that identification methods used to identify organisms for the purpose of Risk Group classification would need to be examined carefully, since some of the methods used do not cover more than just a small spectrum of organisms. This is also linked with a lack of experts trained in this area, a topic also discussed with the VAAM Fachgruppe “Qualitätssicherung und Diagnostik”. Within the ABAS Expertenpool concerns were also raised about the reliable identification and taxonomy of a genome sequenced strain of *Pseudomonas putida* which has also been used as the basis for the safety level evaluation in the ZKBS.

Systematics is affected by the Convention on Biological Diversity and the recently drafted Nagoya Protocol on Access and Benefit Sharing. The EU and many EU member states have not only signed the Nagoya Protocol, but are actively involved in the process leading to the ratification of the protocol. This will lead to additional laws and regulations that will potentially affect many areas of biological research, especially areas relating to “bioprospecting” (screening for new drugs/enzymes). A number of conferences/meetings have already been held in Germany and the process of public consultation is well under way at the EU level.

The next meeting of the VAAM Fachgruppe “Identifizierung und Systematik” will take place in Tübingen where it is hoped that one will be able to further emphasise the fact that microbial systematic is a significant part of microbiology and that it is more than just naming as many species as possible in the shortest possible time using the minimal amount of data. ■

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Fachgruppe: Hefe

■ Hefen haben eine große Bedeutung in der Biotechnologie, und sie sind als Eukaryoten wichtige Modellorganismen der Zellbiologie. Darüber hinaus nimmt die Bedeutung einiger Hefen als human- und pflanzenpathogene Infektionskeime stetig zu. Die Fachgruppe Hefe fasst die Mitglieder der VAAM zusammen, die mit Hefen als Mikroorganismus an diesen Fragestellungen arbeiten und umfasst zurzeit 69 Mitglieder.

Aufgrund der großen Hefetagung „Yeast Genetics and Molecular Biology“ im Sommer 2011 in Olsztyn, Polen, fand kein gesondertes Treffen der Fachgruppe Hefe in 2011 statt. Bei der Tagung in Tübingen wird die Fachgruppe ein Mini-Symposium mit acht Referenten durchführen. Schwerpunktthemen sind dabei: Membranen und Endozytose, RNA und Ribosomen, Biotechnologie und andere Hefen.

Für 2013 wird unter Mitwirkung der Fachgruppe Hefe erstmalig seit 1976 die internationale Hefekonferenz wieder in Deutschland (Frankfurt/Main) stattfinden. ■

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Fachgruppe: Regulation und Signaltransduktion in Prokaryoten

Stressantwort und Stressanpassung sind essentiell für das Überleben einzelliger Organismen. Im Laufe der Evolution haben sich ausgeklügelte Signalnetzwerke entwickelt, die Sensorik sowie nachfolgende transkriptionelle, translationale, posttranslationale Regulationsmechanismen umfassen und niedermolekulare Signalmoleküle einbeziehen. Die Fachgruppe trägt dieser Breite der Regulationsmechanismen Rechnung, indem jährlich während der VAAM-Tagung Symposien organisiert werden, die verschiedene Aspekte der Regulation und Signaltransduktion beleuchten. Die Themenbreite reicht von der Bakteriellen Zellbiologie (2007), Signaltransduktion und Proteinphosphorylierung (2008), zu den Triggerenzymen (2009), der Zell-Zell-Kommunikation (2010) bis zu den Second Messengern (2011). Gleichzeitig unterstützen diese Symposien Initiativen zur Gründung neuer Forscherverbünde. Der neue DFG-Schwerpunkt „Phenotypic heterogeneity and sociobiology of bacterial populations" ist dafür ein gutes Beispiel.

Für das Symposium während der VAAM-Tagung 2012 in Tübingen haben wir das Thema „Bacterial receptors and signaling" gewählt. Das Symposium, das zusammen mit der Fachgruppe „Mikrobielle Pathogenität" organisiert wird, wird in diesem Jahr sechs NachwuchswissenschaftlerInnen die Möglichkeit geben, ihre neuesten Daten zu dieser Thematik vorzustellen.

Eine weitere wichtige Aktivität der Forschergruppe sind die im zweijährigen Turnus stattfindenden Sommerschulen zum Thema „Mechanismen der Genregulation". Die interaktive Diskussion der NachwuchswissenschaftlerInnen steht im Vordergrund dieser Veranstaltungen. Diese Sommerschulen sind eine direkte Fortsetzung des traditionellen „Plasmidsymposiums". Die 29. Sommerschule findet vom 3. bis 5. Oktober 2012 in Wartaweil am Ammersee (in der Nähe von München) statt und wird dankenswerter Weise von Thorsten Mascher, München, organisiert.

Auf diesem Wege möchte ich Sie herzlich zur nächsten Mitgliederversammlung einladen, die im direkten Anschluss an das Fachgruppensymposium in Tübingen stattfinden wird.

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Mitgliederversammlungen der Fachgruppen
General Meetings of the Special Groups

Montag, 19.03.2012, 17:00

Struktur und Mikroskopie	**Hörsaal N8**
Symbiotische Interaktionen	**Hörsaal N4**

Montag, 19.03.2012, 19:00

Identifizierung und Systematik	**Hörsaal N1**

Montag, 19.03.2012, 19:30

Mikrobielle Pathogenität	**Hörsaal N7**
Regulation und Signaltransduktion	**Hörsaal N5**
Fungal Biology and Biotechnology	**Hörsaal N2**
Umweltmikrobiologie	**Hörsaal N3**

Dienstag, 20.03.2012, 16:30

Funktionelle Genomanalyse	**Hörsaal N8**

Industrial session: Microbiology in industrial application

Tuesday, 20.03.2012
13:00–14:30

Lecture hall N5

13:00–13:10
Dr. Gerhard Schmid
Wacker Chemie AG, Munich
Microbiology in industrial application – An Overview

13:10–13:30
Dr. Oskar Zelder
BASF, Ludwigshafen
Fermentation Products – Employing Nature's Biosynthetic Power

13:30–13:50
Dr. Jochen Förster
DTU Biosustain, Technical University of Denmark, Kgs. Lyngby
Towards bio-based production of chemicals

13:50–14:10
Dr. Jürgen Eck
B.R.A.I.N., Zwingenberg
From Biodiversity to Designer Bugs: Bio-inspired engineering of producer microorganisms

14:10–14:30
Dr. Günter Wich
Wacker Chemie AG, Munich
Wacker's cysteine process – rational design based on systems biology

Microbiology at the University of Tübingen

■ Today, Microbiology and Infection Biology is one of the five research priorities of the University of Tübingen, a result of steadily increasing activities in the past 50 years. Around 1960, the German Research Council (Wissenschaftsrat) advised the government to expand university education, which in 1964 resulted in the foundation of the first chair of Microbiology at the University of Tübingen. Hans Zähner, who led a group engaged in screening for new antibiotics at the Swiss Federal Institute of Technology of Zürich (ETH Zurich), was appointed Professor. He established a large group, which worked on the isolation of biologically active secondary metabolites from bacteria and fungi. He was for most of the time speaker of two Collaborative Research Centres (SFBs), "Chemical Biology of Microorganisms" and "Microbial Fundamentals of Biotechnology", which lasted for 30 years. Scientists from different disciplines – microbiology, biochemistry, organic chemistry, pharmaceutical biochemistry, and botany – worked together on related subjects. In 1974, Volkmar Braun was appointed to a newly established second chair of microbiology, named "Microbiology/Membrane Physiology". In 1987, Friedrich Götz was appointed to a newly founded chair of "Microbial Genetics" and Bernhard Schink became successor to Hans Zähner, who continued to work as Fiebiger Professor until he retired in 1994. In 1994, Wolfgang Wohlleben followed Bernhard Schink on the chair of "Microbiology/Biotechnology". The chair of "Medical Microbiology" was filled in 2000 with Ingo Autenrieth and 2003 Andreas Peschel was appointed Professor of "Cellular and Molecular Microbiology" in the Department of Medical Microbiology and Hygiene. In 2007, Volkmar Braun retired and took over a position as Max Planck Fellow at the MPI for Developmental Biology. Karl Forchhammer became his follower on the chair now named "Microbiology/Organismic Interactions". In 2008, Andreas Kappler was appointed Professor for Geomicrobiology, a foundation of the Stifterverband in the Geosciences Department. The close cooperation between the Natural Science Microbiology and the Medical Microbiology led to the establishment of the Interfaculty Institute of Microbiology and Infection Medicine (IMIT) in 2009. Rüdiger Hampp, chair of "Physiological Ecology of Plants" and engaged in rhizosphere bacteria research, and Dominik Hartl, heading the Pediatric Infections Deseases and Immunology Section and exploring infections in cystic fibrosis patients, joined the IMIT .

A view accross the conference location "Morgenstelle" towards the "Schwäbische Alb"

Actually the SFB 766 "The Bacterial Cell Envelope: Structure, Function and Infection Interface" and the Transregio TRR34 "Pathophysiology of Staphylococci in the Postgenomic Era" join groups from the IMIT with groups from the Max-Plank-Institute for Developmental Biology (Andrei Lupas, Dirk Linke), groups from the departments of Chemistry, Pharmacy and Biochemistry and the University Hospital. Recently, a new research-training group (GRK1708) on "Molecular Principles of Bacterial Survival Strategies" was granted and will start in April 2012. In addition, numerous projects funded by the DFG, the BMBF, the State of Baden-Württemberg, and the EU are established in the area of Microbiology.

Prof. Dr. Wolfgang Wohlleben
IMIT, Department of Microbiology/ Biotechnology

The major aim of the groups working in the Microbiology/Biotechnology section is to understand the secondary metabolism (in particular the antibiotic biosynthesis and resistance) in actinomycetes and its integration in the general metabolism and biology of these filamentous soil bacteria. To achieve this goal, we are concentrating on the following topics:

Glycopeptides (vancomycin, teicoplanin) are used as drugs of last resort to combat life-threatening infections caused by multiresistant Gram-positive bacteria. We have elucidated the glycopeptide biosynthesis, self resistance and precursor supply in the producer *Amycolatopsis balhimycina* in order to generate novel derivatives and to devise strategies for yield optimisation (Evi Stegmann).

For the development of novel anti-infectives we employ the „Tübinger Stammsammlung" (initially built up by Prof. Zähner), which contains potent secondary metabolite producers. Using this collection we have solved the biosynthesis of complex antibiotics such as kirromycin and lysolipin in order to modify these compounds to make them potential drugs. In addition, a bioinformatic pipeline was developed which allows the identification of biosynthetic pathways in whole genome sequences and their use for prediction of the enzymatic reactions and the structures of the compounds (Tilmann Weber).

As a model system to study the evolution of secondary metabolite specific functions the phosphinothricin-tripeptide producer *Streptomyces viridochromogenes* is used. Its biosynthesis includes steps which greatly resemble reactions of the primary metabolism such as an aconitase. These investigations revealed that the aconitase has – in addition to its catalytic function – an important regulatory role

in the differentiation of actinomycetes by binding to specific mRNAs (Yvonne Mast).

Secondary metabolite production strongly depends on precursor supply from the primary metabolism. One of the limiting nutrients is nitrogen. We are, therefore, investigating how the nitrogen metabolism in *Streptomyces* is transcriptionally regulated by the orphan response regulator GlnR, which controls both, ammonium supply and assimilation (Agnieszka Bera).

During their life cycle the bacteria undergo a complex morphological and physiological differentiation. We investigate cytoskeletal elements (such as Mre-proteins), which play an important role in spore formation in *Streptomyces*, but not in vegetative growth. An important parameter in the evolution of secondary metabolite pathways is the acquisition and rearrangement of biosynthetic genes. Thereby gene transfer is a crucial step. We could show that this process in *Streptomyces* is completely different to transfer processes in other bacteria. Gene transfer is mediated by only one protein which transfers double-stranded DNA from a donor into a recipient (Günther Muth).

New actinomycetes strains are isolated and taxonomically characterized in the group of Prof. Hans-Peter Fiedler. These strains are screened for the presence of novel secondary metabolites by HPLC-DAD-MS. After fermentation scale-up and isolation of the compounds they are tested for their biological activities.

The Junior Research Group of Christoph Mayer analyses the bacterial sugar metabolism with an emphasis on cell wall metabolism. The research aims to understand how the cell wall is reshaped during growth and differentiation.

Prof. Dr. Friedrich Götz
IMIT, Department of Microbial Genetics

Friedrich Götz was appointed Professor for „Microbial Genetics“ at the University Tübingen in 1987. He has a broad interest in staphylococci, particularly in studying molecular processes involved in virulence and survival strategies. Areas of research are: Biosynthesis of lantibiotics; biofilm-formation, which plays a crucial role in chronic and implant-associated infections; unraveling physiological alterations in a biofilm community contributing to antibiotic tolerance; activation of the innate immunity and staphylococcal resistance mechanisms to immune response (lysozyme and defensins) – we demonstrated by using defined mutants that lipoproteins and peptidoglycan are in *Staphylococcus aureus* the major players; finally, we are studying the cell separation, which is in staphylococci catalyzed by the major autolysin (Atl).

The group of Ralph Bertram aims at elucidating molecular mechanisms of dormant bacteria, such as staphylococcal persister cells. These are characterized by a temporarily confined growth cessation despite nutritional abundance. Methods to select for *S. aureus* persisters by antibiotic challenge were recently established and respective cells are currently subject to comprehensive transcriptional, metabolic and morphological characterization. We are particularly interested in defining the roles of toxin-antitoxin (TA) systems in bacterial growth control or cell death and in dissecting their regulatory mechanisms, some of which are controlled by small non-coding RNAs. The development of molecular genetic tools to facilitate recombination and inducible gene expression in firmicutes is another focus of our group.

The junior research group of Ute Bertsche studies biosynthesis of the peptidoglycan sacculus, the stress bearing layer of the bacterial cell envelope, which consists of glycan strands cross-linked by short peptides. It is a major target for antibacterial treatment, but the knowledge about its synthesis and the interplay of the involved proteins is still very limited, especially in Gram-positive cocci. The penicillin-binding proteins (PBPs), which catalyze the last steps in peptidoglycan polymerization, as well as proteins of the SEDS-family (shape, elongation, division, sporulation) which are thought to flip the precursor across the cytoplasmic membrane, are the enzymes of interest. In addition the peptidoglycan composition of various antibiotic non-susceptible strains is characterized to unravel their resistance mechanism.

Prof. Dr. Karl Forchhammer
IMIT, Department of Micorbiology/ Organismic Interactions

This research unit focuses on the molecular biology of cyanobacteria and on nitrogen-stress acclimation responses in bacteria in general. Cyanobacteria are dominating bacteria in the biosphere, inhabiting almost all illuminated ecosystems, where they play pivotal roles in the global cycles of C, N and O. Furthermore, they have a widely unexplored potential for biotechnological applications. We use unicellular cyanobacteria for fundamental research addressing questions of metabolic regulation and signaling. The P_{II} signal transduction proteins coordinate C/N metabolism with the energy status of the cells. How the P_{II} proteins work and function at the molecular level is our primary research goal, which goes beyond cyanobacteria and which has led to milestone discoveries. Furthermore, we are interested in the physiological responses of cyanobacteria towards nutrient deprivation and other conditions of imbalanced metabolism, which cause fundamental reprogramming of cellular processes with biotechnological impact.

A second focus is on cellular differentiation in multicellular cyanobacteria of the order *Nostocales.* Their filaments are composed of hundreds of mutually dependent vegetative cells and regularly spaced N_2-fixing heterocysts, exchanging metabolites and signaling molecules. Furthermore, they can differentiate spore-like cells and motile filaments. In one line of research, the synthesis of the heterocyst-specific cell-wall is addressed, where we have identified the first ATP-driven efflux pump for glycolipids. In a second project, cell-wall amidases, which we identified to play a key role in morphogenesis and development of *Nostoc* strains, are investigated as a model system for multicellular development in prokaryotes.

Prof. Dr. Rüdiger Hampp
IMIT, Department of Physiological Ecology of Plants

The release of organic compounds from plant roots into the surrounding soil forms the basis for a versatile community of microorganisms that distinctly influences the productivity of plants. Fungi and bacteria are a major constituent of this ecosystem, which has been termed “rhizosphere”. They modify growth and distribution of symbiotic and pathogenic organisms or improve vitality and resistance of plants against pathogen attacks.

Soil bacteria belonging to the actinomycetes, especially the streptomycetes, are commonly found in the rhizosphere of plants. Due to the release of secondary metabolites, they are capable of exhibiting beneficial as well as detrimental effects towards plants, including promotion of symbiosis, improved growth and biotic and abiotic stress resistance but also enhanced disease susceptibility and repressed defence responses.

We investigate the specific and selective effects of soil streptomycetes towards the development of symbiosis between plants and fungi (mycorrhiza) but also with respect to

plant disease resistance against pathogenic micro-organisms such as root rot producing fungi (*Heterobasidion* sp. on Norway spruce), seed decomposing fungi (*Neofusicoccum* sp. on Araucariaceae; co-operation with Brazilian and Australian research groups) as well as leaf pathogenic fungi (*Alteranaria brassicicola* on *Arabidopsis thaliana*). We focus on both molecular responses of target organisms and bacterial and fungal effector molecules (collaboration with Hans-Peter Fiedler, IMIT) which contribute to the observed interaction patterns.

Prof. Dr. Ingo Autenrieth
IMIT, Department of Medical Microbiology and Hygiene

The Department of Medical Microbiology and Hygiene is involved in research, teaching of students (human and dental medicine, molecular medicine, biology etc.) and is running the infectious disease laboratory diagnostic unit of the University Hospital Tübingen.

The question addressed by the various research groups is how bacterial pathogens by means of virulence factors and how microbial products act on host cells and the host immune system, particularly how virulence factors contribute to immune evasion.

The research focus is host-microbe interactions particular at mucosal surfaces and the role of the microbiota in mucosal immune responses and inflammation. The aim is to unravel basic mechanisms of pathogenesis of bacterial infections and bacteria-triggered inflammatory processes in order to understand the principles of host susceptibility for and resistance to infections, respectively, and in consequence to find novel approaches of therapeutic intervention. The approach includes mainly cellular microbiology and molecular biology methods as well as experimental animal infection models.

University Hospital, Department of Medical Microbiology

While some of the research groups are working on the pathogenesis of *Yersinia enterocolitica* infections focusing on the type three secretion system and outer membrane proteins of *Y. enterocolitica*, and analyze how bacterial proteins affect the host immune response, particularly dendritic cells (Autenrieth I., Autenrieth S., Bohn, Schütz); others address *Staphylococcus* infections (Weidenmaier, Wolz, Liese) and infections in cystic fibrosis patients (Döring). Furthermore, the role of commensal bacteria of the intestinal tract in intestinal immune homeostasis, inflammatory bowel diseases and susceptibility to gastrointestinal infections is under investigation (Frick).

Prof. Dr. Andreas Peschel
IMIT, Department of Cellular and Molecular Microbiology Section

Andreas Peschel's lab investigates the biology and pathogenicity of *Staphylococcus aureus*, a bacterial species capable of two profoundly different life styles – as an unremarkable constituent of the human nasal microbiota or as a major pathogen causing severe disseminated infections along with high mortality. The processes governing the successful colonization of the nose and subsequent infection of sterile tissues represent major topics of the lab's four research teams. Cell wall teichoic acids, complex glycopolymers at the staphylococcal cell surface and their role in mediating adhesion to host epithelial cells and lectins are in the focus of Guoqing Xia's team. Bernhard Krismer and colleagues study the metabolic adaptation of *S. aureus* to life in the nose and the staphylococcal interference with competing microbes. The modulation of local and systemic inflammation by *S. aureus* peptide toxins, the 'phenol-soluble modulins', is investigated by the team headed by Dorothee Kretschmer. The fourth team (Christoph Ernst et al) explores how *S. aureus* copes with antimicrobial peptides released by host leukocytes and epithelial cells and how the corresponding mechanisms contribute to *S. aureus* antibiotic resistance.

Prof. Dr. Andreas Kappler
Center for Applied Geosciences, Department of Geomicrobiology

The Geomicrobiology Group at the Center of Applied Geosciences focuses on the interactions of microorganisms and minerals in modern and ancient environments. Iron minerals largely determine the fate of many nutrients (e.g. trace metals, phosphate) as well as the environmental behaviour of harmful (in)organic pollutants. Microbial Fe(II)-oxidizing and Fe(III)-reducing communities play a key role in iron-mineral formation and transformation. Studying both biological molecular mechanisms and geochemical conditions are crucial to the identification, localization and quantification of these biogeochemical processes. Therefore, together with the associated junior research groups of Molecular Microbial Ecology (S. Behrens), Microsensors and Biogeochemical Modelling (C. Schmidt) and the affiliated Emmy-Noether research group for Analytical Microscopy (M. Obst), we combine microscopic, spectroscopic and molecular techniques with geochemical analysis in order to understand modern and ancient iron biogeochemistry and its environmental impact. In more detail, our projects comprise i) biogeochemistry and molecular ecology of microbial Fe cycling, ii) physiology, genetics, high-resolution imaging and spectroscopic analysis of microbial Fe(II) oxidation and Fe biomineralization, iii) microbial magnetite formation, iv) humic substances as electron shuttles in biogeochemical redox processes, v) mechanisms of deposition and diagenesis of Fe minerals in Precambrian Banded Iron Formations, vi) consequences of microbial Fe(II) oxidation and Fe(III) reduction for the environmental fate of Cd and As in soil-plant-microbe systems and in As drinking water filters, vii) microbial formation and degradation of halogenated organic compounds, viii) impact of biochar addition on soil microbial community composition, microbial N-cycling and contaminant transformation.

Prof. Dr. Lutz Heide
Pharmaceutical Institute, Department of Pharmaceutical Biology

The Department of Pharmaceutical Biology, headed by Prof. L. Heide, works on the discovery and development of new antibiotics from actinomycetes. Aminocoumarin antibiotics like novobiocin are potent inhibitors of bacterial gyrase and topoisomerase IV, and novobiocin has been introduced into human therapy (Albamycin). The group of L. Heide has identified the biosynthetic gene clusters of several aminocoumarin antibiotics and elucidated the functions of nearly all of the genes contained therein. This knowledge is used for the production of new antibiotics by targeted genetic manipulation of the gene clusters followed by heterologous expression in engineered host strains. New antibiotics are also generated by mutasynthesis, chemoenzymatic synthesis and methods of synthetic biology. The Heide group also works on prenylated phenazines and naphthoquinones from actinomycetes and carries out biochemical and structural studies on a new class of microbial prenyltransferases with aromatic substrates (ABBA prenyltransferases).

PD Dr. Bertolt Gust has established an independent research area focusing on MraY translocase inhibitors, i.e. inhibitors of the first step in the membrane cycle of reactions during peptidoglycan biosynthesis. The Gust group has sequenced and analyzed the biosynthetic gene clusters of caprazamycins, liposidomycins, napsamycins and pacidamycins and is now utilizing them for the production of new antibiotics.

Dr. Kristian Apel establishes a research program on the regulation of secondary metabolism in Streptomyces, focussing on their regulation during heterologous expression in *Streptomyces coelicolor*. Further projects include the use of inducible promoters to activate silent gene clusters, as well as combinatorial biosynthesis using artificial gene operons which are being combined in a new SuperCos-based vector.

Prof. Dr. Stephanie Grond
Institute of Organic Chemistry, Department of Natural Product Analysis/ Biomolecular Chemistry

Microbial natural products act as indispensable drugs in human medicine, research agents in biochemistry, and as important compounds in agricultural applications. They function as regulators of proteins or cell mechanisms, and often they are the first indispensable step to make molecular biochemical investigations even possible. A primary goal of our natural product research is to get profound knowledge of the character of compounds from the secondary metabolism of microorganisms. Our interest is to develop new natural chemical structures towards biochemical tools. Our current focus is on streptomycetes and fungi. Methods of chemistry, microbiology, and molecular biology comprise cultivation, chemical analysis, spectroscopy (MS, NMR), and chemical syntheses.

Prof. Dr. Volkmar Braun,
Prof. Dr. Andrei Lupas, PD Dr. Dirk Linke
Max-Planck Institute for Developmental Biology, Department I and Associated Research Groups

The Department of Prof. Andrei Lupas studies Protein Evolution. Protein folding is too complex to have arisen de novo. We are pursuing the hypothesis that folded proteins evolved by fusion and recombination from an ancestral set of peptides, which emerged as cofactors in the context of RNA dependent replication and catalysis (the 'RNA world'). These peptides were initially optimized to become structured on RNA scaffolds and their assembly into longer polypeptide chains led to scaffold-independent folding as an emerging property. Systematic studies should allow a description of this peptide set in the same way in which ancient vocabularies have been reconstructed from the comparative study of modern languages. To this end we apply cutting-edge bioinformatic tools (available through toolkit.tuebingen.mpg.de), as well as a range of experimental techniques, including protein biochemistry, spectroscopy, and structural biology. Our model organisms are typically bacteria and archaea. We also focus on a number of other questions relating to the evolution of proteins. We would like to understand how changes in protein structure can lead to the emergence of new biological functionality. Here we study primarily the mechanisms by which type I receptors transduce signals across membranes and AAA ATPases disassemble, unfold and translocate proteins. We also explore the genetic processes that lead to changes in the topology of protein folds or the evolution of entirely new folded proteins. Here, a particularly powerful phenomenon is the repetition of polypeptide segments, from short peptides that lead to fibrous folds, such as coiled coils, over supersecondary structures that lead to solenoidal or toroidal folds, such as propellers, to entire domains that lead to segmented structures, such as trimeric autotransporter adhesins in Gram-negative bacteria.

The associated microbiology research groups in the department study different aspects of bacterial transporters, adhesins, and toxins.

The Linke group is interested in the onset of infection, which is mostly determined by the ability of pathogens to adhere to host cells. How are adhesins synthesized and exported to the cell surface? How is the synthesis regulated? What are the host cell binding partners of different adhesins? Pathogens protect themselves from the environment by capsule and biofilm formation, by binding and inactivation of components of the host immune system, or by variable expression of surface molecules to evade detection. What surface molecules are produced, how and when are they synthesized, and what are their protective advantages?

The Braun group currently focuses on an imported toxin that requires a helper protein (chaperone, prolyl cis-trans isomerase) to kill cells. The toxin is imported by an energy-coupled process which involves energy transfer from the cytoplasmic membrane into the outer membrane. The energy-transferring protein complex is studied to understand the energy harvesting and transfer mechanisms. In addition, export of a protein cytotoxin is investigated which is coupled to activation of the toxin. Export across the outer membrane belongs to the two-partner secretion systems. The export and activation domains in the exporting protein and the mechanism of activation of the exported toxin are characterized.

Prof. Dr. Joachim Schulz and
Prof. Dr. Klaus Hantke
Pharmaceutical Institute, Department of Pharmaceutical Industry

The Schultz/Hantke group has a lasting interest in the global second messenger cyclic AMP. Most bacteria possess adenylyl cyclases which are similar to mammalian congeners; many of them are membrane delimited. For the most part, the regulation of their activity in the cytoplasm remains enigmatic. Intracellular cyclic AMP binds to regulator proteins governing gene transcription. Our major goal is to mechanistically elucidate transmembrane signal transduction. Bacterial membrane-anchored adenylyl cyclases have 2 to 6 transmembrane spans which carry more or less pronounced periplasmic domains, probably orphan receptors. The C-terminal

transmembrane span connects via distinct linker regions to the catalytic domain. Similar designs of signaling proteins are histidine kinases of the two component systems and methyl accepting chemotaxis proteins. The chemotaxis receptor Tsr of *E. coli* is anchored by two transmembrane spans. It has a periplasmic domain which senses serine. On the cytoplasmic side a ubiquitous signal-transducing element (HAMP domain; >14,000 data base entries) connects to the output domain. In chimeras with the Tsr receptor and various adenylyl cyclases serine regulates cyclase activity. Such chimeras are suitable for examination of individual domains and their interplay in intramolecular signalling. Thus, we investigate *in vitro* and *in vivo* the mechanism of signaling through the HAMP domain and through a subsequent linker, termed S-helix, which connects to the output cyclase. The sign of the cytoplasmic signal, cyclase inhibition or activation, may be controlled by the S-helix. In an experimental extension, we attempt to understand the workings of strings of HAMP domains, e.g. a tandem HAMP from *Natronomonas pharaoni*.

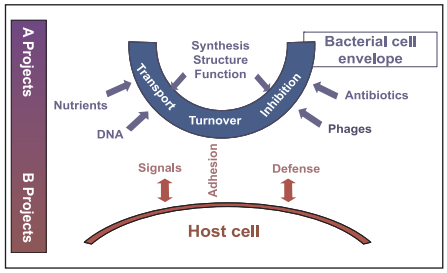
SFB 766: The Bacterial Cell Envelope: Structure, Function and Infection Interface

Speaker: Wolfgang Wohlleben, Tübingen

The Collaborative Research Center 766 (SFB 766) was initiated in 2007 and was recently extended until 2015. In 21 projects the interdisciplinary network of researchers aims to gain a more in-depth understanding of the structure and biosynthesis of the bacterial cell envelope and its interactions with the environment. The projects are carried out at various departments of the Faculty of Sciences, the University Hospital and at the Max-Planck-Institute of Developmental Biology.

The bacterial cell envelope has a decisive function in basic bacterial processes such as morphogenesis, uptake and secretion, sensitivity or resistance towards antimicrobial agents, as well as in microbe-host interactions including bacterial adherence, immune recognition and evasion. Research in the SFB766 is dedicated to expanding our understanding of the structure, function, and the biosynthesis of the bacterial cell envelope and its interaction with mammalian or plant hosts or with bacteriophages.

The investigation of these questions is organized in two tightly integrated Sections A and B. Central subject in Section A is the synthesis, turnover and chemical composition of peptidoglycan, lipids, and polysaccharides in Gram-positive bacteria. In addition, transport of molecules (such as DNA and antibiotics) and transduction of signals across the cell wall are studied. Moreover, the interactions of the cell envelope with the environment are investigated. These studies provide crucial insights into the structure and function of the bacterial cell wall. Section B addresses the role of individual components of the bacterial cell envelope in microbe-host interaction in bacterial colonization and infection. A particular emphasis is put on proteins of the bacterial surface that interact with eukaryotic host cells as well as on the recognition of envelope components by the immune systems of human, murine and plant cells.



Scheme of the enhanced concept of the SFB766.

The interdisciplinary consortium characterizes the cell wall with a combination of methods including cellular microbiology, structural biology, molecular genetics, biochemistry and bioinformatics. The results will enhance our understanding of bacterial physiology and pathogenicity and contribute to the identification of new antimicrobial agents, vaccines and diagnostics aiming to the development of new preventive and curative health care strategies.

Research Training Group (Graduiertenkolleg) 1708: Molecular principles of bacterial survival strategies

Speaker: Karl Forchhammer, Tübingen

The new research training group "Molecular principles of bacterial survival strategies", granted by the DFG in 2011, will be launched in April 2012. It addresses the question, how bacteria maintain viability in a hostile environment. Bacteria grow exponentially only under optimal conditions. But in many habitats, they are exposed to adverse conditions, arresting their growth or challenging their viability. This selective pressure throughout evolution resulted in the acquisition of elaborated strategies to withstand and overcome unfavourable conditions. These processes are therefore fundamental for bacteria to protect their niches and colonize new habitats, an issue of highest relevance in bacterial ecology, physiology and medicine, e.g. for understanding the dispersal of bacterial pathogens and for the development of new antimicrobial drugs. 13 projects are devoted to the investigation of bacterial survival strategies involving maintenance-metabolism, detoxification, repair pathways and protective substances and structures. The research training group provides a new interdisciplinary research platform for fundamental microbiolocial research in Tübingen. Groups from the IMIT (Interfaculty Institute for Microbiology and Infection Medicine Tübingen), from Geomicrobiology, from the Organic Chemistry and the Max-Planck Institute for Developmental Biology contribute to the program. An accompanying study program shall mediate expert knowledge as well as professional skills, establishing the research training group as a central part of the PhD training within the priority cluster "Microbiology and Infection Biology" of the University Tübingen and the University Medical Centre.

Transregional Collaborative Research Centre 34
Pathophysiology of staphylococci in the post-genomic era

Speaker: Michael Hecker, Greifswald

Deputy Coordinator: Friedrich Götz, Tübingen

Five microbiologists and biochemists from Tübingen (Götz, Peschel, Stehle,Weidenmaier, and Wolz) are members of the Transregional Collaborative Research Centre 34: „Pathophysiology of staphylococci in the post-genomic era". Coordinator: Prof. Dr. Michael Hecker, Institut für Mikrobiologie, Universität Greifswald. Besides Greifswald and Tübingen, groups of the Universities Münster and Würzburg are also involved. While the knowledge of bacterial genomes is rapidly increasing, the increasing gap in understanding of physiology, virulence or host-pathogen interaction is lagging. The aim of the TR-SFB is to fill this gap of knowledge by transferring the expertise in proteomics, metabolomics, structural genomics and bioinformatics to the groups more specialized in physiology, genetics and infection biology of staphylococci. In a concerted effort we want to breath more life into the genome sequences.

Sunday, 18 March 2012

	Lecture hall N6	Lecture hall N7	Lecture hall N5	Lecture hall N3	Lecture hall N2	Lecture hall N4	Lecture hall N9	Lecture hall N1	Lecture hall N8
15:30–16:00	Welcome Addresses								
	p. 32								
16:00–17:00	**Public Lecture**								
	p. 32								
	Coffee break/Industrial exhibition								
17:30–18:30	**Plenary Session I** Soil Microbiology								
	p. 32								

Monday, 19 March 2012

	Lecture hall N6	Lecture hall N7	Lecture hall N5	Lecture hall N3	Lecture hall N2	Lecture hall N4	Lecture hall N9	Lecture hall N1	Lecture hall N8
08:30–10:30		Molecular Structure and Biochemistry p. 42	Bacterial cell surface, antibiotics and novel therapy approaches p. 42	Microbial processes involved in carbon and nitrogen cycling p. 42	Metabolic Regulation and Signalling: Signals and Perception p. 43	Molecular Biology p. 43	From the Genome to the Product p. 44	Adaptation of microorganisms to chemical and physical stressors p. 44	
	Coffee break/Industrial exhibition								
11:00–12:45	**VAAM Honorary Award** **Plenary Session II** Microbial Pathogenicity p. 32								
12:45–14:15	Lunch break/Industrial exhibition			**13:00–14:30** Berufsbilder im Bereich Qualität und Diagnostik p. 35	Lunch break/Industrial exhibition				
14:15–15:15	**Plenary Session III** Cell Envelope p. 34								
15:15–17:30	**15:45–17:15** Karriere-Symposium p. 14	Poster Session I (odd poster numbers)/Coffee break/Industrial exhibition							
17:30–19:30		Cell walls, membranes, and lipids in microbial pathogenesis p. 37	Bacterial receptors and signaling p. 38	What makes carbon sources difficult for microbes to degrade? p. 35	Fungi go omics – Fungal systems biology approaches in the postgenomics era p. 36	Symbiotic Interactions p. 39	Membranes and Endocytosis p. 40	Systematics – Quo vadis? p. 40	**17:00–17:30** Annual Meeting Special Group Struktur und Mikroskopie p. 35

☐ Short Lecture ■ Special Group Mini-Symposia

Tuesday, 20 March 2012

	Lecture hall N6	Lecture hall N7	Lecture hall N5	Lecture hall N3	Lecture hall N2	Lecture hall N4	Lecture hall N9	Lecture hall N1	Lecture hall N8
08:30–10:30		Enzymology/ Biotechnology p. 45	Bacteria host cell interaction and host response p. 45	Structural and Regulatory Aspects p. 45	Responses and Pathways p. 46	Geomicrobiology and Environmental Microbiology p. 46	Virulence factors, function and regulation p. 47	Membrane transport and dynamics p. 47	
	Coffee break/Industrial exhibition								
11:00–12:00	**Plenary Session IV** Bacterial Differentiation p. 33								
12:00–13:00	**Plenary Session V** Metabolic Regulation p. 33								
13:00–14:30	Lunch break/ Industrial exhibition		Industrial Session p. 22	Lunch break/Industrial exhibition					
14:30–15:30	**Plenary Session VI** Secondary Metabolites p. 34								
15:30–17:30	Poster Session II (even poster numbers)/Coffee break/Industrial exhibition								**16:30–17:00** Annual Meeting Special Group Funktionelle Genomanalyse
17:30–19:30	VAAM Annual General Meeting/PhD Awards (18:30)								
19:30	**Mensa Morgenstelle**								
	Mixer								

☐ Short Lecture

Wednesday, 21 March 2012

	Lecture hall N6	Lecture hall N7	Lecture hall N5	Lecture hall N3	Lecture hall N2	Lecture hall N4	Lecture hall N9	Lecture hall N1
09:00–11:00		Enviromental Microbiology	Microbial Pathogenicity IV/ Human Microbiota	Physiology: Redox systems and cytochormes	Fermentation Studies/Microbial Survival Strategies	New aspects of bacterial cell differentiation	Cell wall synthesis and maintenance	Fungal Genetics and Physiology
		p. 48	p. 48	p. 48	p. 49	p. 49	p. 50	p. 50
Coffee break/Industrial exhibition								
11:30–11:45	Poster Awards							
11:45–13:15	**Plenary Session VII** Microbial Survival/Microbiota Strategies							
	p. 34							
13:15–13:30	Closing Remarks							

☐ Short Lecture

CONFERENCE PROGRAMME

VAAM 2012 Jahrestagung Tübingen (18.03.–21.03.2012)

Sunday, 18.03.2012

15:30–16:00 **Welcome Addresses** — **Lecture hall N6**

W. Wohlleben
Eberhard Karls Universität Tübingen, Department of Microbiology/Biotechnology, Tübingen, Germany

H. Müther
Eberhard Karls Universität Tübingen, Department of Physics, Tübingen, Germany

PUBLIC LECTURE — **Lecture hall N6**
Chair: Friedrich Götz

16:00–17:00 ISV01: **K.-H. Schleifer**
Technische Universität München, Mikrobiologie, München, Germany
Die verborgene Welt der Bakterien und ihre Bedeutung für das Leben auf der Erde

17:00–17:30 Coffee break/Industrial exhibition

PLENARY SESSION: SOIL MICROBIOLOGY — **Lecture hall N6**
Chair: Bernhard Schink

17:30 ISV02: **R. Conrad**
Max-Planck-Institut für terrestrische Mikrobiologie, Biogeochemie, Marburg, Germany
From microorganisms to the atmosphere: flooded soils and the methane cycle

18:00 ISV03: **A. Kappler**
Eberhard Karls Universität, Geomicrobiology, Center for Applied Geosciences, Tübingen, Germany
Physiology, mechanisms and habitats of microbial Fe(II) oxidation

Monday, 19.03.2012

08:00–19:30 Industrial exhibition — **Ground/1st floor**

08:30–10:30 **Short lectures** (see page 42) — **various**

10:30–11:00 Coffee break/Industrial exhibition — **Ground/1st floor**

11:00 **VAAM Honorary Award Session**
ISV04: **S. Albers**
Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany
Assembly and function of archaeal surface structures

PLENARY SESSION: MICROBIAL PATHOGENICITY — **Lecture hall N6**
Chair: Andreas Peschel

11:45 ISV05: **T. F. Meyer**
Max-Planck-Institut für Infektionsbiologie, Molekulare Biologie, Berlin, Germany
Current views on the role as well as the fate of host cells during infection

12:15 ISV06: **R. Rappuoli**
Novartis Vaccines and Diagnostics Srl, Siena, Italy
Vaccines to address the needs of a 21st century society

12:45–14:15 Lunch break/Industrial exhibition — **Cafeteria/Ground & 1st floor**

13:00–14:30 **Fachgruppe Qualitätssicherung und Diagnostik** (see page 35) — **Lecture hall N3**
Berufsbilder im Bereich Qualität und Diagnostik

CONFERENCE PROGRAMME

VAAM 2012 Jahrestagung Tübingen (18.03.–21.03.2012)

PLENARY SESSION: CELL ENVELOPE **Lecture hall N6**
Chair: Volkmar Braun

14:15 ISV07: **A. Peschel**
Universitätsklinikum Tübingen, Medical Microbiology and Hygiene Department, Tübingen, Germany
Teichoic acids in Gram-positive cell wall function and host interaction

14:45 ISV08: **J. Tommassen**
Utrecht University, Molecular Microbiology, Utrecht, Netherlands
Out of the iron age: the battle for zinc

15:15–17:30 Coffee break/Industrial exhibition **Ground/1st floor**

15:15–17:30 Poster Session I (odd poster numbers) **Ground/1st floor & cafeteria**

15:45–17:15 **Karrieresymposium** (see page 14) **Lecture hall N6**
Vorstellung vielfältiger Berufsbilder in den Biowissenschaften

17:30–19:30 **Special Groups Mini Symposia** (see page 35) **various**

various General Meetings of the Special Groups (see page 22) **various**

Tuesday, 20.03.2012

08:00–19:00 Industrial exhibition **Ground/1st floor**

08:30–10:30 **Short lectures** (see page 45) **various**

10:30–11:00 Coffee break/Industrial exhibition **Ground/1st floor**

various General Meetings of the Special Groups (see page 22) **various**

PLENARY SESSION: BACTERIAL DIFFERENTIATION **Lecture hall N6**
Chair: Andreas Kappler

11:00 ISV09: **J. Errington**
Newcastle University, Centre for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences, Newcastle upon Tyne, United Kingdom
Origins and proliferation of L-form (cell-wall deficient) *Bacillus subtilis*

11:30 ISV10: **L. Søgaard–Andersen**
Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany
Positive regulation of cell division site positioning in bacteria by a ParA protein

PLENARY SESSION: METABOLIC REGULATION **Lecture hall N6**
Chair: Karl Forchhammer

12:00 ISV11: **A. Ninfa**
University of Michigan Medical School, Department of Biological Chemistry, Ann Arbor, MI, USA
Integration of signals in the regulation of bacterial nitrogen assimilation

12:30 ISV12: **J. Stülke**
Georg-August-Universität, Allgemeine Mikrobiologie, Göttingen, Germany
Signalling in biofilm formation of *Bacillus subtilis*

13:00–14:30 Lunch break/Industrial exhibition **Cafeteria/Ground & 1st floor**

13:00–14:30 **Industrial session** (see page 22) **Lecture hall N5**
Microbiology in industrial application

CONFERENCE PROGRAMME

VAAM 2012 Jahrestagung Tübingen (18.03.–21.03.2012)

PLENARY SESSION: SECONDARY METABOLITES — **Lecture hall N6**
Chair: Lutz Heide

14:30	ISV13: **W. Wohlleben** *Eberhard Karls Universität Tübingen, Department of Microbiology/Biotechnology, Tübingen, Germany* Glycopeptide Antibiotics: Biosynthesis, Resistance, Evolution	
15:00	ISV14: **P. Leadlay** *University of Cambridge, Department of Biochemistry, Cambridge, United Kingdom* The biosynthetic engineering of polyketide drugs	
15:30–17:30	Coffee break/Industrial exhibition	**Ground/1st floor**
15:30–17:30	Poster Session II (even poster numbers)	**Ground/1st floor & Cafeteria**
17:30–18:30	**VAAM Annual General Meeting** (see page 14)	**Lecture hall N6**
ca. 18:30	**PhD Awards** Sponsored by BASF SE, Sanofi Aventis Deutschland GmbH, Bayer Schering Pharma, New England Biolabs GmbH, Evonik Degussa GmbH	**Lecture hall N6**
ca. 19:30	Mixer	**Mensa/Cafeteria Morgenstelle**

Wednesday, 21.03.2012

08:30–12:00	Industrial exhibition	**Ground/1st floor**
09:00–11:00	**Short lectures** (see page 48)	**various**
11:00–11:30	Coffee break/Industrial exhibition	**Ground/1st floor**
11:30–11:45	**Poster Awards** Sponsored by MorphoSys AG Chair: Rüdiger Hampp	**Lecture hall N6**

PLENARY SESSION: MICROBIAL SURVIVAL STRATEGIES — **Lecture hall N6**
Chair: Ingo Autenrieth

11:45	ISV15: **A. Walker** *Wellcome Trust Sanger Institute, Pathogen Genomics Group, Hinxton, UK* Suppression of *Clostridium difficile* disease and transmission by the intestinal microbiota	
12:15	ISV16: **D. Oesterhelt** *Max-Planck-Institut für Biochemie, Membranbiochemie, Martinsried, Germany* Systems biology of halophilic archaea	
12:45	ISV17: **R. Proctor** *Emeritus Professor of Medical Microbiology/ Immunology and Medicine, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA* Microbial survival strategies: *Staphylococcus aureus* as a highly effective surviver	
13:15–13:30	**Closing Remarks**	**Lecture hall N6**

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 19, 13:00–14:30

Special Group: Quality Assurance & Diagnostics (Qualitätssicherung & Diagnostik)

Topic: Berufsbilder im Bereich Qualität und Diagnostik – Einstiegs- und Karrieremöglichkeiten

Organisation: S. Prowe, Beuth Hochschule für Technik, Fachbereich V – Studiengang Biotechnologie, Berlin, Germany; A. Seiffert-Störiko, Sanofi-Aventis Deutschland GmbH, Frankfurt-Höchst, Germany
Lecture hall N3

QDV1-FG 13:00 **S. Pering*, B. Gerten**
Merck KGaA, Merck Millipore Biomonitoring, Darmstadt, Germany
Development of hygiene monitoring media with non-animal origin – bachelor thesis written in industry

QDV2-FG 13:15 **S. Wickert**
Beuth Hochschule für Technik Berlin, FB V „Life Sciences and Technology" Studiengang Biotechnologie, AG Prof. Dr. Prowe, Berlin, Germany
Erfahrungen als EMbaRC-Stipendiatin bei der BCCM/LMG in Gent – Nutzen für das eigene Forschungsprojekt

QDV3-FG 13:30 **A. Kolk*[1], U. Jäckel[2], E. Martin[2], J. Schäfer[2], G. Schneider[1]**
[1]Institut für Arbeitsschutz (IFA) der Deutschen Gesetzlichen Unfallversicherung (DGUV), Sankt Augustin, Germany
[2]Bundesanstalt für Arbeitsschutz und Arbeitsmedizin (BAuA), Berlin, Germany
Das Berufsfeld des Mikrobiologen in einer Behörde – Diagnostik im Rahmen der biologischen Arbeitssicherheit

QDV4-FG 13:45 **M. Egert**
Hochschule Furtwangen University, Department of Mechanical and Process Engineering, Villingen-Schwenningen, Germany
From academia to industry, and back: Microbiological research to make life easier, better and more beautiful

Diskussion

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

Special Group: Structure and Microscopy (Struktur und Mikroskopie)

Organisation: H. Engelhardt, Max-Planck-Institut für Biochemie, Martinsried, Germany
Lecture hall N8

17:00 **Annual Meeting of the Special Group Struktur und Mikroskopie**

Special Group: Environmental Microbiology (Umweltmikrobiologie)

Topic: What makes carbon sources difficult for microbes to degrade?

Organisation: R. Meckenstock, Helmholtz Zentrum München, Institut für Grundwasserökologie, Neuherberg, Germany; B. Morasch, University of Tübingen, Center for Applied Geoscience (ZAG), Environmental Mineralogy and Chemistry, Tübingen, Germany
Lecture hall N3

EMV1-FG 17:30 **Invited Speaker: F. Widdel**
Max Planck Institute for Marine Microbiology, Bremen, Germany
Degradation of organic carbon by microorganisms – do we know the 'rules' and limits?

EMV2-FG 18:00 **Invited Speaker: F. Hammes**
Eawag, Microbiology, Dübendorf, Switzerland
Characterising oligotrophic bacterial growth with flow cytometry

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

EMV3-FG 18:30 **A. Schwedt*[1], M. Seidel[1,2], T. Dittmar[1,2], M. Simon[2], V. Bondarev[1], S. Romano[1], G. Lavik[1], H.N. Schulz-Vogt[1]**
[1]Max Planck Institute for Marine Microbiology, Microbiology, Ecophysiology Group, Bremen, Germany
[2]Carl von Ossietzky University of Oldenburg, Institute of Chemistry and Biology of the Marine Environment, Oldenburg, Germany
Substrate use of extremely oligotrophic bacteria

EMV4-FG 18:42 **M. Kästner*, A. Miltner**
Helmholtz-Centre for Environmental Research, Environmental Biotechnology, Leipzig, Germany
Microbial degradation of organic compounds (natural compounds, xenobiotics, and pesticides) and the formation of soil organic matter and biogenic non-extractable (or bound) residues

EMV5-FG 18:54 **B. Morasch*, S.B. Haderlein**
University of Tuebingen, Center for Applied Geoscience (ZAG), Tuebingen, Germany
What keeps microorganisms from eating emerging contaminants? – A study on the corrosion inhibitor benzotriazole

EMV6-FG 19:06 **Y. Liu[1,2], S.-J. Liu[2], H.L. Drake[1], M. Horn*[1]**
[1]University of Bayreuth, Ecological Microbiology, Bayreuth, Germany
[2]Chinese Academy of Sciences, State Key Laboratory of Microbial Resources, Institute of Microbiology, Beijing, China
Phenoxyacetic acids – what soil microbes can handle ether-linkages in soil?

EMV7-FG 19:18 **C. Eberlein*[1], H. Mouttaki[2], R. Meckenstock[2], M. Boll[1]**
[1]University of Leipzig, Institute of Biochemistry, Leipzig, Germany
[2]Helmholtz Center Munich, German Research Center for Environmental Health, Institute of Groundwater Ecology, Munich, Germany
A new function for an old yellow enzyme: dearomatizing naphthoyl-CoA reductase, a key enzyme in anaerobic naphthalene degradation

Special Group: Fungal Biology and Biotechnology (Experimentelle Mykologie)
Topic: Fungi go *omics* – Fungal systems biology approaches in the postgenomics era

Organisation: S. Krappmann, University of Würzburg, Research Center for Infectious Diseases, Würzburg, Germany; V. Meyer, Technische Universität Berlin, Institute for Biotechnology, Dept. of Applied and Molecular Microbiology, Berlin, Germany
Lecture hall N2

FBV1-FG 17:30 **Invited Speaker: C. Sagt**
DSM Biotechnology Center, Beijerinck Laboratory, Delft, Netherlands
Applied genomics in an industrial setting

FBV2-FG 18:00 **B.M. Nitsche*[1], T.R. Jrgensen[1,2], V. Meyer[2,3], A.F.J. Ram[1,2]**
[1]Leiden University, Institute of Biotechnology, Leiden, Netherlands
[2]Kluyver Centre for Genomics of Industrial Fermentation, Delft, Netherlands
[3]Berlin University of Technology, Institute of Biotechnology, Berlin, Germany
The carbon depletion response of *Aspergillus niger* during submerged cultivation

FBV3-FG 18:15 **P. Olbermann*[1], S. Tarazona[2], H. Irmer[3], C. Jöchl[4], D. Turras[5], A. Di Pietro[5], H. Haas[4], G.H. Braus[3], A. Conesa[2], S. Krappmann[1]**
[1]Universität Würzburg, Zentrum für Infektionsforschung, Würzburg, Germany
[2]Centro de Investigacion Príncipe Felipe, Bioinformatics and Genomics Department, Valencia, Spain
[3]University of Göttingen, Institute for Microbiology and Genetics, Göttingen, Germany
[4]Innsbruck Medical University, Division of Molecular Biology, Innsbruck, Austria
[5]University of Cordoba, Department of Genetics, Cordoba, Spain
Blood is a very special fluid – the transcriptome of *Aspergillus fumigatus* in response to human blood

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

FBV4-FG 18:30 **K. Kroll*[1,2], M. Vödisch[1,2], M. Roth[3], A.A. Brakhage[1,2], O. Kniemeyer[1,2]**
[1]Hans-Knöll-Institute, Department of Molecular and Applied Microbiology, Jena, Germany
[2]Friedrich-Schiller-University Jena, Jena, Germany
[3]Hans-Knöll-Institute, Department of Bio Pilot Plant, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany
Proteomic profiling of the short-term response of *Aspergillus fumigatus* to hypoxic growth conditions

FBV5-FG 18:45 **A. Kühn*[1], H. Kusch[1], C. Hoppenau[1], K. Michels[2], I. Feussner[2], B. Voigt[3], D. Becher[3], M. Hecker[3], S.A. Braus-Stromeyer[1], G.H. Braus[1]**
[1]Georg-August Universität Göttingen, Institut für Mikrobiologie und Genetik, Göttingen, Germany
[2]Georg-August Universität Göttingen, Abteilung Biochemie der Pflanze, Albrecht-von-Haller-Institut für Pflanzenwissenschaften, Göttingen, Germany
[3]Ernst-Moritz-Arndt-Universität Greifswald, Institut für Mikrobiologie, Greifswald, Germany
Differential analysis of intra- and extra-cellular proteomes of *Verticillium longisporum* during biotrophic and saprophytic growth

FBV6-FG 19:00 **M. Navarro-Gonzalez*, M. Arndt, M. Zomorrodi, A. Majcherczyk, U. Kües**
Georg-August-Universität Göttingen, Molekulare Holzbiotechnologie und technische Mykologie, Göttingen, Germany
Regulation of fruiting body formation in *Coprinopsis cinerea*

FBV7-FG 19:15 **J. Bormann*, N. Van Thuat, W. Schäfer**
University of Hamburg, Biocenter Klein Flottbek, Department of Molecular Phytopathology and Genetics, Hamburg, Germany
The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum*

Followed by **Annual Meeting of the Special Group Experimentelle Mykologie**

Special Group: Microbial Pathogenicity (Mikrobielle Pathogenität)
Topic: Cell walls, membranes, and lipids in microbial pathogenesis

Organisation: A. Peschel, Universitätsklinikum, Medical Microbiology and Hygiene Department, Tübingen, Germany
Lecture hall N7

MPV1-FG 17:30 **S. Weber*, S. Dolinsky, I. Haneburger, H. Hilbi**
Max von Pettenkofer Institute, Bacteriology, Munich, Germany
Modulation of phosphoinositide metabolism by *Legionella spp.*

MPV2-FG 17:50 **M. Türck*, G. Bierbaum**
University of Bonn, Institute of Medical Microbiology, Immunology and Parasitology (IMMIP), Bonn, Germany
The YycFG (WalRK/VicRK) two-component regulatory system of *Staphylococcus aureus* and its capability to sense changes in membrane fluidity

MPV3-FG 18:10 **K. Zeth*[1], V. Kozjak-Pavlovic[2], M. Faulstich[2], O. Kepp[2], T. Rudel[2]**
[1]University of Tübingen, ZMBP, Tübingen, Germany
[2]University of Würzburg, Department of Microbiology, Würzburg, Germany
Structure and function of the PorB porin from disseminating *N. gonorrhoeae*

MPV4-FG 18:30 **S. Brown[1], G. Xia*[2], L. G. Luhachack[3], J. Campbell[1], T. Meredith[1], C. Chen[1], V. Winstel[2], C. Gekeler[2], J. E. Irazoqui[3], A. Peschel[2], S. Walker[1]**
[1]Department of Microbiology and Immunobiology, Harvard Medical School, Boston MA, USA
[2]Cellular and Molecular Microbiology Section, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Tübingen. Germany
[3]Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, Boston, USA
Methicillin Resistance in *Staphylococcus aureus* depends on β-O-GlcNAcylation of Wall Teichoic Acids

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

MPV5-FG 18:50 **D. Asam*[1], S. Mauerer[1], E. Walheim[2], B. Spellerberg[1]**
[1]University Hospital Ulm, Institute for Medical Microbiology and Hygiene, Ulm, Germany
[2]University Ulm, Ulm, Germany
Identification of β-haemolysin encoding genes in *Streptococcus anginosus*

MPV6-FG 19:10 **F. Glowinski[1], K. Paprotka[2], M. Grosz[3], B. Sinha[3], M. Fraunholz*[2]**
[1]Max Planck Institute for Infection Biology, Dept. Molecular Biology, Berlin, Germany
[2]University of Würzburg , Chair of Microbiology, Biocenter, Würzburg, Germany
[3]Univeristy of Würzburg, Institute of Hygiene and Microbiology, Würzburg, Germany
Alternative pathways of phagosomal escape of *Staphylococcus aureus*

Followed by **Annual Meeting of the Special Group Mikrobielle Pathogenität**

Special Group: Regulation and Signal Transduction (Regulation und Signaltransduktion)
Topic: Bacterial receptors and signaling

Organisation: K. Jung, Ludwig-Maximilians Universität München, Biozentrum, Department Biologie I, Bereich Mikrobiologie, Martinsried, Germany
Lecture hall N5

RSV1-FG 17:30 **S. Hunke*[1], V.S. Müller[1], K. Tschauner[1], P. Scheerer[1,2]**
[1]Universität Osnabrück, Molekulare Mikrobiologie, Osnabrück, Germany
[2]Charité – Universitätsmedizin Berlin, Institut für Medizinische Physik und Biophysik (CC2), Berlin, Germany
Signal recognition and transmission by the CpxAR-two component system

RSV2-FG 17:50 **J. Natarajan*, J. Schultz**
University of Tübingen, Department of Pharmaceutical Biochemistry, Tübingen, Germany
Mechanism of signal transfer by the tandem hamp domain from *Natronomonas pharaonis*

RSV3-FG 18:10 **D.J. Leslie[1], S. Dintner[2], A. Starón[2], T. Petri[3], F. Kalamorz[1], G.M. Cook[1], T. Mascher[2], S. Gebhard*[2]**
[1]University of Otago, Department of Microbiology and Immunology, Dunedin, New Zealand
[2]Ludwig-Maximilians-Universität München, Department of Biology I, Microbiology, Planegg-Martinsried, Germany
[3]Ludwig-Maximilians-Universität München, Department of Informatics, Research and Teaching Unit Bioinformatics, München, Germany
Signalling within resistance modules against peptide antibiotics – regulatory interplay between ABC-transporters and two-component systems

RSV4-FG 18:30 **J. Lassak*, S. Ude, T. Kraxenberger, K. Jung**
Ludwig-Maximilians-University Munich, Munich Center for integrated Protein Science (CiPSM) at the Department of Biology I, Microbiology, Martinsried, Germany
The one-component regulator CadC of *E. coli* is a targetof the elongation factor P

RSV5-FG 18:50 **F. Zähriger[1], E. Lacanna[2], U. Jenal[1], T. Schirmer[1], A. Böhm*[2]**
[1]University of Basel, Biozentrum, Basel, Germany
[2]University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany
A Zn^{2+}-sensory diguanylate-cyclase from *Escherichia coli*

RSV6-FG 19:10 **N. Masloboeva*[1], L. Reutimann[1], P. Stiefel[1], H. Hennecke[1], S. Mesa[2], H.-M. Fischer[1]**
[1]ETH Zurich, Institute of Microbiology, Zurich, Switzerland
[2]Estación Experimental del Zaidín, Department of Soil Microbiology and Symbiotic Systems, Granada, Spain
Reactive oxygen species-inducible ECF s factors of *Bradyrhizobium japonicum*

Followed by **Annual Meeting of the Special Group Regulation und Signaltransduktion**

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

Special Group: Symbiotic Interactions (Symbiotische Interaktionen)

Topic: Symbiotic Interactions

Organisation: U. Hentschel Humeida, Julius-Maximilians-Universität Würzburg, Lehrstuhl Botanik II, Julius-von-Sachs-Institut für Biowissenschaften, Würzburg, Germany; A. Schwiertz, Institut für Mikroökologie, Herborn, Germany
Lecture hall N4

17:00 **Annual Meeting of the Special Group Symbiotische Interaktionen**

Theme I: Lower Metazoan Symbioses

SIV1-FG 17:30 **Invited Speaker: V. Weis**
Oregon State University, Department of Zoology, Corvallis, USA
The regulation of cnidarian-dinoflagellate mutalisms: in sickness and in health

SIV2-FG 18:00 **V. Gloeckner*[1], S. Schmitt[1], N. Lindquist[2], U. Hentschel[1]**
[1]University of Wuerzburg, Julius von Sachs Institute for Biological Sciences, Wuerzburg, Germany
[2]University of North Carolina at Chapel Hill, Institute of Marine Sciences, Chapel Hill, USA
Amount, activity and mode of transmission of microbial symbionts associated with the Caribbean sponge *Ectyoplasia ferox*

Theme II: Low versus High Microbial Diversity Symbioses

SIV3-FG 18:15 **J. Zimmermann*[1], J.M. Petersen[1], J. Ott[2,3], N. Musat[1], N. Dubilier[1]**
[1]Max Planck Institute for Marine Microbiology, Molecular Ecology, Symbiosis Group, Bremen, Germany
[2]University of Vienna, Department of Molecular Ecology, Vienna, Austria
[3]University of Vienna, Department of Marine Biology, Vienna, Austria
Highly specific nematode symbioses from the North Sea and the benefits of harbouring ectosymbionts

SIV4-FG 18:30 **T. Köhler*, C. Dietrich, A. Brune**
Max Planck Institute for Terrestrial Microbiology, Department of Biogeochemistry, Marburg, Germany
Digesting the diversity – evolutionary patterns in the gut microbiota of termites and cockroaches

Theme III: Symbiont Metabolism and Ecology

SIV5-FG 18:45 **A. Siegl*[1], B.S. Sixt[1], C. Müller[2], M. Watzka[3], A. Richter[3], P. Schmitt-Kopplin[2], M. Horn[1]**
[1]University of Vienna, Department of Microbial Ecology, Vienna, Austria
[2]Helmholtz-Zentrum Muenchen – German Research Center for Environmental Health, Institute of Ecological Chemistry, Department of Molecular BioGeoChemistry and Analytics, Neuherberg, Germany
[3]University of Vienna, Department of Chemical Ecology and Ecosystem Research, Vienna
Metabolic activity of the obligate intracellular amoeba symbiont *Protochlamydia amoebophila* in a host-free environment

SIV6-FG 19:00 **H.-P. Grossart*[1,2], C. Dziallas[1], K.T. Tang[1,3]**
[1]Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany
[2]University of Potsdam, Institute for Biochemistry and Biology , Potsdam, Germany
[3]College of William & Mary, Virginia Institute of Marine Science, Gloucester, USA
Bacteria-zooplankton interactions: a key to understanding bacterial dynamics and biogeochemical processes in lakes?

Theme IV: Symbioses Factors

SIV7-FG 19:15 **B. Kranzusch[1], S. Albert[1], K. Kunze[1], M. Kunke[1], A. Weiss[1], E. Szentgyörgyi[1], O. Walser[2], M. Göttfert[1], S. Rossbach*[1]**
[1]Technische Universität Dresden, Institut für Genetik, Dresden, Germany
[2]Western Michigan University, Department of Biological Sciences, Kalamazoo, USA
Efflux pumps and TetR-like regulators in rhizobial interactions with plants

SIV8-FG 19:30 **D. Zhurina, M. Gleisner, C. Westermann, J. Schützner, C.U. Riedel***
University of Ulm, Institute of Microbiology and Biotechnology, Ulm, Germany
Host colonization of bifidobacteria – from genome sequence to protein function

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

Special Group: Systematics (Systematik)

Topic: Quo vadis?

Organisation: B. Tindall, Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany
Lecture hall N1

SYV1-FG 17:30 **Invited Speaker: I.C. Sutcliffe**
Northumbria University, School of Life Sciences, Newcastle upon Tyne, United Kingdom
The road ahead for microbial systematics: raising our game in the post-genomic era

SYV2-FG 18:00 **Invited Speaker: B.J. Tindall**
Leibniz Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany
The purpose of prokaryote systematics; clarifying muddy waters

SYV3-FG 18:30 **V. Salman*[1], R. Amann[1], A.-C. Girnth[1], L. Polerecky[1], J. Bailey[2], S. Høgslund[3], G. Jessen[4], S. Pantoja[4], H.N. Schulz-Vogt[1]**
[1]Max Planck Institute for Marine Microbiology, Bremen, Germany
[2]University of Minnesota – Twin Cities, Department of Geology and Geophysics, Minneapolis, MN, USA
[3]Aarhus University, Department of Biological Sciences, Aarhus, Denmark
[4]University of Concepción, Department of Oceanography and Center for Oceanographic Research in the Eastern South Pacific, Concepción, Chile
A single-cell sequencing approach to the classification of large, vacuolated sulfur bacteria

SYV4-FG 18:45 **K. Voigt*[1,2], P. M. Kirk[3]**
[1]Leibniz Institute for Natural Product Research and Infection Biology, Jena Microbial Resource Collection, Jena, Germany
[2]University of Jena, Dept. Microbiology and Molecular Biology, Jena, Germany
[3]CABI UK Centre, Surrey, United Kingdom
A phylogeny-compliant revision of the systematics for the basal fungal lineages: Chytridiomycota and Zygomycota

19:00 **Discussion**

Followed by **Annual Meeting of the Special Group Systematik**

Special Group: Yeast (Hefe)

Topic: Membranes and endocytosis

Organisation: K.-D. Entian, Biozentrum Niederursel, Institut für Mikrobiologie, Frankfurt a.M., Germany
Lecture hall N9

YEV1-FG 17:30 **D. Rapaport**
University of Tuebingen, Interfaculty Institute of Biochemistry, Tübingen, Germany
New and old tricks in the biogenesis of mitochondrial outer membrane proteins

YEV2-FG 17:45 **E. Gießelmann, J. Dausend, B. Becker, M.J. Schmitt***
Saarland University, Department of Biosciences (FR 8.3), Molecular & Cell Biology, Saarbrücken, Germany
Mechanistic insight into receptor endocytosis and endosomal A/B toxin trafficking in yeast

YEV3-FG 18:00 **A. Hackmann, T. Gross, C. Baierlein, H. Krebber***
University of Göttingen, Institute for Microbiology and Genetics, Department Molecular Genetics, Göttingen, Germany
The conjunction of mRNA export and translation

YEV4-FG 18:15 **J. Fundakowski[1], M. Schmid[2], C. Genz[1], S. Lange[2], R.-P. Jansen*[1]**
[1]Eberhard-Karls-Universität Tübingen, Interfaculty Institute for Biochemistry, Tübingen, Germany
[2]Ludwig-Maximilians-Universität München, GeneCenter, Munich, Germany
Localization of mRNAs and endoplasmic reticulum in budding yeast

Mini-Symposia of the Special Groups: Monday, March 19, 17:30–19:30

YEV5-FG 18:30 **K.-D. Entian*, B. Meyer**
Johann Wolfgang Goethe University, Cluster of Excellence: Macromolecular Complexes and Institute for Molecular Biosciences, Frankfurt a.M., Germany
Eukaryotic Ribosome Biogenesis: Analysis of the Nucleolar Essential Yeast Nep1 Protein and Mutations Causing the Human Bowen-Conradi Syndrome

YEV6-FG 18:45 **C. Schorsch, E. Boles***
Johann Wolfgang Goethe University, Institute of Molecular Biosciences, Frankfurt a.M., Germany
High-level production of tetraacetyl phytosphingosine (TAPS) by combined genetic engineering of sphingoid base biosynthesis and L-serine availability in the non-conventional yeast *Pichia ciferrii*

YEV7-FG 19:00 **S. Fischer[1], E. Sieber[2], Z. Zhang[2], J. Heinisch[3], C. von Wallbrunn*[1]**
[1]Geisenheim Research Center, Department of Microbiology and Biochemistry, Geisenheim, Germany
[2]Hochschule RheinMain, Fachbereich Geisenheim, Geisenheim, Germany
[3]University of Osnabrück, Department of Genetics, Faculty of Biology, Osnabrück, Germany
The genetics of ester synthesis in *Hanseniaspora uvarum* during winemaking

YEV8-FG 19:15 **R. Schaffrath*[1,2], C. Bär[1,2], D. Jablonowski[1,2]**
[1]Universität Kassel, Institut für Biologie, Abteilung Mikrobiologie, Kassel, Germany
[2]University of Leicester, Department of Genetics, Leicester, Germany
Feel me, thrill me, kill me – when *K. lactis* meets *S. cerevisiae*

Mini-Symposia of the Special Groups: Tuesday, March 20, 16:30

Special Group: Funktionelle Genomanalyse

Organisation: H.-P. Klenk, Leibniz-Institut DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
Germany

16:30 **Annual Meeting of the Special Group Funktionelle Genomanalyse**

Monday, March 19, 08:30–10:30

Open Topics I: Molecular structure and biochemistry

Lecture hall N7

Chair: Sonja Albers
Co-Chair: Dirk Linke

OTV001
08:30
*N.A. CHRIST, S. BOCHMANN, D. GOTTSTEIN, E. DUCHARDT-FERNER, U. HELLMICH, S. DÜSTERHUS, P. KÖTTER, P. GÜNTERT, K.-D. ENTIAN, J. WÖHNERT
The first structure of a LanI protein, SpaI: The protein conferring autoimmunity against the lantibiotic subtilin in *Bacillus subtilis* reveals a novel fold

OTV002
08:45
*S. BOCHMANN, N. CHRIST, P. KÖTTER, S. DÜSTERHUS, J. WÖHNERT, K.-D. ENTIAN
Analysis of SpaI-mediated lantibiotic immunity in *Bacillus subtilis*

OTV003
09:00
*T. STAUFENBERGER, J.F. IMHOFF, A. LABES
First crenarchaeal chitinase detected in *Sulfolobus tokodaii*

OTV004
09:15
H. GULDAN, F.-M. MATYSIK, M. BOCOLA, R. STERNER, *B. PATRICK
A novel biosynthetic pathway for the synthesis of Archaea-type ether lipids in Bacteria

OTV005
09:30
*D. LINKE, S. SHAHID, M. HABECK, B. BARDIAUX, B. VAN ROSSUM
De novo structure of the membrane anchor domain of the trimeric autotransporter YadA by solid-state NMR spectroscopy

OTV006
09:45
*T. NEINER, K. LASSAK, A. GHOSH, S. HARTUNG, J.A. TAINER, S.-V. ALBERS
Biochemical and structural analysis of FlaH, a component of the crenarchaeal flagellum

OTV007
10:00
*L. SIMON, M. ULBRICH, J. RIES, H. EWERS, P.L. GRAUMANN
Subcellular positioning of a DNA-binding protein through constraint movement

OTV008
10:15
*N. DE ALMEIDA, H. WESSELS, W. MAALCKE, J. KELTJENS, M. JETTEN , B. KARTAL
Protein complexes involved in the electron transport chain of anammox bacteria

MP I: Bacterial cell surface, antibiotics and novel therapy approaches

Lecture hall N5

Chair: Volkhard Kempf
Co-Chair: Erwin Bohn

MPV001
08:30
H. BRÖTZ-OESTERHELT, *A. MUELLER, D. MUENCH, Y. SCHMIDT, K. REDER-CHRIST, G. SCHIFFER, G. BENDAS, H. GROSS, H.-G. SAHL, T. SCHNEIDER
The lipodepsipeptide empedopeptin inhibits cell wall biosynthesis through Ca^{2+}-dependent complex formation with peptidoglycan precursors

MPV002
08:45
I. BLEIZIFFER, K. MCAULAY, G. XIA, M. HUSSAIN, G. POHLENTZ, A. PESCHEL, S.J. FOSTER, G. PETERS, *C. HEILMANN
The *Staphylococcus aureus* plasmin-sensitive protein Pls is a glycoprotein

MPV003
09:00
*B. OESTERREICH, R. KONTERMANN, C. ERCK, U. LORENZ, K. OHLSEN
From target to therapy – Expression and characterization of an anti-staphylococcal antibody

MPV004
09:15
*C. SZAGUNN, T.A. WICHELHAUS, V.A.J. KEMPF, S. GÖTTIG
Antibiotic resistance and pathogenicity of NDM-carrying *Acinetobacter baumannii*

MPV005
09:30
*P. OBERHETTINGER, M. SCHÜTZ, J. LEO, D. LINKE, I. AUTENRIETH
Intimin and invasin export their C-terminus to the bacterial cell surface using an inverse mechanism compared to classical autotransport

MPV006
09:45
*A. GRÜTZNER, M. ROHDE, G.S. CHHATWAL, S.R. TALAY
Analysis of the interaction of invasive M1 *Streptococcus pyogenes* with human endothelial cells

MPV007
10:00
*C. WEBER, K. KRAUEL, A. GREINACHER, S. HAMMERSCHMIDT
Lipopolysaccharides of Gram-negative bacteria contribute to the creation of heparin-induced thrombocytopenia-eliciting antibodies by binding and conformationally altering platelet factor 4

MPV008
10:15
*D. KRETSCHMER, N. NIKOLA, M. DUERR, M. OTTO, A. PESCHEL
***Staphylococcus epidermidis* and *Staphylococcus aureus* Quorum Sensing System agr Regulates Formyl Peptide Receptor 2 Ligand Secretion and thereby the Activation of the Innate Immune System**

Soil Microbiology I: Microbial processes involved in carbon and nitrogen cycling

Lecture hall N3

Chair: Sebastian Behrens

SMV002
08:30
*Z. JAKOB, H. SYLVIA, B. ALEXANDRE, T. SONIA, C. FRANZ
Distribution, diversity, and activity of anaerobic ammonium oxidizing bacteria in soils

SMV003
08:45
*K. PALMER, M.A. HORN
Denitrification activity of a new and diverse denitrifier community in a pH neutral fen soil in Finnish Lapland is nitrate limited

Monday, March 19, 08:30–10:30

SMV004
09:00
*P.S. DEPKAT-JAKOB, G.G. BROWN, S.M. TSAI, M.A. HORN, H.L. DRAKE
Emission of Denitrification-derived Nitrogenous Gases by Brazilian Earthworms

SMV005
09:15
B. ZHU, G. VAN DIJK, C. FRITZ, M.S.M. JETTEN, *K.F. ETTWIG
Anaerobic methane oxidizers prevent methane emissions from a minerotrophic peatland

SMV006
09:30
*A. GITTEL, J. BARTA, I. LACMANOVA, V. TORSVIK, A. RICHTER, S. OWENS, J. GILBERT, C. SCHLEPER, T. URICH
Microorganisms affecting the stabilisation of soil organic carbon in cryoturbated soils of the Siberian Arctic

SMV007
09:45
*C. SCHURIG, R. SMITTENBERG, J. BERGER, F. KRAFT, S.K. WOCHE, M.-O. GÖBEL, H.J. HEIPIEPER, A. MILTNER, M. KÄSTNER
Could bacterial residues be an important source of SOM? – A case study from a glacier forefield

SMV008
10:00
*A. STACHETER, H.L. DRAKE, S. KOLB
Methanol Consumption by Methylotrophs in Temperate Aerated Soils

SMV017
10:15
*S. GWOSDZ, J. WEST, D. JONES, K. SMITH, M. KRÜGER
Effects of elevated CO_2 concentrations on microbial ecosystem at the artificial test site ASGARD, England

Metabolic Regulation and Signalling I: Signals and perception

Lecture hall N2

Chair: Kirsten Jung
Co-Chair: Joachim E. Schultz

RSV001
08:30
*J. WITAN, G. UNDEN
Functional interaction of the *Escherichia coli* transporters DctA and DcuB with the sensor kinase DcuS

RSV002
08:45
*Y. GÖPEL, B. REICHENBACH, K. PAPENFORT, C. SHARMA, J. VOGEL, B. GÖRKE
YhbJ – a novel RNA binding protein functions as mediator of signal transduction in the hierarchically acting GlmYZ sRNA cascade

RSV003
09:00
*H. ANTELMANN, B.K. CHI, P. WAACK, K. GRONAU, D. BECHER, D. ALBRECHT, W. HINRICHS, R.J. READ, G. PALM
Structural insights into the redox-switch mechanism of HypR, a disulfide stress-sensing MarR/DUF24-family regulator of *Bacillus subtilis*

RSV004
09:15
*O. FOKINA, K. FORCHHAMMER
How the P_{II} protein from *Synechococcus* integrates metabolic with energy signals to control its targets

RSV005
09:30
*J. OBERENDER, M. BOLL
Post-translational modification determines the substrate specificity of a carboxylic acid-coenzyme A ligase

RSV006
09:45
*D. ESSER, J. REIMANN, T.K. PHAM, S.V. ALBERS, P.C. WRIGHT, B. SIEBERS
Hot signal transduction in the thermoacidophilic creanarchaeum *Sulfolobus acidocaldarius*

RSV007
10:00
I. HITKOVA, C. MANSKE, S. BRAMEYER, K. SCHUBERT, C. HARMATH, S. LINNERBAUER, S. JOYCE, D. CLARKE, *R. HEERMANN
A novel LuxR-based cell-to-cell communication system in the entomopathogen *Photorhabdus luminescens*

RSV008
10:15
*A. KESSLER, U. SCHELL, C. HARRISON, H. HILBI
α-Hydroxyketone-mediated signal transduction in *Legionella pneumophila*

Open Topics II: Molecular biology

Lecture hall N4

Chair: Julia Fritz-Steuber
Co-Chair: Jörg Soppa

OTV009
08:30
*T. WALDMINGHAUS, C. WEIGEL, K. SKARSTAD
Replication fork movement and methylation governs SeqA binding to the *Escherichia coli* chromosome

OTV010
08:45
*H. GRÖNHEIM, W. STEFFEN, J. STEUBER
Translocation of sodium ions by the ND5 subunit of mitochondrial complex I from the yeast *Yarrowia lipolytica*

OTV011
09:00
*V. SALMAN, R. AMANN, D. SHUB, H. SCHULZ-VOGT
Large and frequent introns in the 16S rRNA genes of large sulfur bacteria

OTV012
09:15
*S. LAASS, J. KLEIN, D. JAHN, P. TIELEN
Regulation of anaerobic respiratory pathways in *Dinoroseobacter shibae*

OTV013
09:30
S. BOSCHI BAZAN, G. GEGINAT, T. BREINIG, M.J. SCHMITT, *F. BREINIG
Influence of subcellular antigen localization within different yeast genera on the activation of ovalbumin-specific CD8 T lymphocytes

Monday, March 19, 08:30–10:30

OTV014
09:45
*B. PETERS, M. MIENTUS, D. KOSTNER, W. LIEBL, A. EHRENREICH
The quest for new oxidative catalysts: Expression of metagenomic membrane-bound dehydrogenases from acetic acid bacteria in *Gluconobacter oxydans*

OTV015
10:00
*C. WITHARANA, L. HOU, C. LASSEK, V. ROPPELT, G. KLUG, E. EVGUENIEVA-HACKENBERG
Growth phase dependent changes of the RNA degrading exosome in *Sulfolobus solfataricus*

OTV016
10:15
*S.L. GARCIA, A. SRIVASTAVA, H.-P. GROSSART, T. MCMAHON, R. STEPANAUSKAS, A. SCZYRBA, T. WOYKE, S. BARCHMANN, F. WARNECKE
Freshwater Actinobacteria acI as revealed by single-cell genomics

Secondary Metabolites I: From the genome to the product

Lecture hall N9

Chair: Elke Dittmann
Co-Chair: Tilmann Weber

MEV001
08:30
*M. KAI, O. GENILLOUD, S. SINGH, A. SVATOŠ
Mass spectrometric analysis of antibiotics from bacteria

MEV002
08:45
*J. DISCHINGER, M. JOSTEN , A.-M. HERZNER, A. YAKÉLÉBA, M. OEDENKOVEN, H.-G. SAHL, J. PIEL , G. BIERBAUM
Mining for new lantibiotic producer in microbial genome sequences

MEV003
09:00
*B. KRAWCZYK, W.M. MÜLLER, P. ENSLE, R.D. SÜSSMUTH
Biosynthesis of class III lantibiotics – *in vitro* studies

MEV004
09:15
*B. BOLL, T. TAUBIZ, L. HEIDE
The Effect of MbtH-like Proteins on the Adenylation of Tyrosine in the Biosynthesis of Aminocoumarin Antibiotics and Vancomycin

MEV005
09:30
*E.M. MUSIOL, T. HÄRTNER, A. KULIK, W. WOHLLEBEN, T. WEBER
KirCI and KirCII, the discrete acyltransferases involved in kirromycin biosynthesis

MEV006
09:45
*Q. ZHOU, H.B. BODE
Investigation of the type II polyketide synthase from Gram-negative bacteria *Photorhabdus luminescence* TT01

MEV007
10:00
*A. ADAM, K.-H. VAN PÉE
Purification and Characterisation of the Flavin-Dependent Monodechloroaminopyrrolnitrin 3-Halogenase from Pyrrolnitrin Biosynthesis

MEV008
10:15
*M. MATUSCHEK, C. WALLWEY, X. XIE, S.-M. LI
EasG and FgaFS are key enzymes in the differentiation of ergot alkaloid biosynthesis in *Claviceps purpurea* and *Aspergillus fumigatus*

Microbial Survival Strategies I: Adaptation of microorganisms to chemical and physical stressors

Lecture hall N1

Chair: Jörg Stülke
Co-Chair: Ralph Bertram

SSV001
08:30
*A. BARTSCH, A. KLINGNER, J. BECKER, C. WITTMANN
Metabolic pathway fluxes of the marine model bacterium *Dinoroseobacter shibae* under changing environmental conditions

SSV002
08:45
*V. BEHRENDS, K.J. WILLIAMS, V.A. JENKINS, B.D. ROBERTSON, J.G. BUNDY
Glucosyl-glycerate is a nitrogen stress-dependent carbon-capacitator in *Mycobacterium smegmatis*

SSV003
09:00
*L. PLATZEN, A. MICHEL, B. WEIL, M. BROCKER, M. BOTT
Flavohemoprotein Hmp of *Corynebacterium glutamicum* is involved in nitrosative stress resistance

SSV004
09:15
A. YAN
Drug efflux as a surviving strategy in response to the anaerobic stress in *E. coli*

SSV005
09:30
*S. MIRIAM, B. AVERHOFF
Metabolic adaptation of *Acinetobacter* to changing environmental conditions

SSV006
09:45
*K. SELL, E.A. GALINSKI
The incompatible solute creatine inhibits bacterial Na^+/H^+ antiporters

SSV007
10:00
*A. KIRSTEN, M. HERZBERG, D.H. NIES
How *Cupriavidus metallidurans* deals with toxic transition metals

SSV008
10:15
*J.-P. OUEDRAOGO, S. HAGEN, V. MEYER
Accept your fate? Defence strategies of yeast and filamentous fungi against the chitin synthase inhibitor AFP

Tuesday, March 20, 08:30–10:30

Open Topics III: Enzymology/Biotechnology

Lecture hall N7

Chair: Garabed Antranikian
Co-Chair: Bernhard Hauer

OTV017
08:30
*O. RASIGRAF, C. VOGT, H.-H. RICHNOW, M.S.M. JETTEN, K.F. ETTWIG
Carbon and hydrogen isotope fractionation during nitrite-dependent anaerobic methane oxidation by *Methylomirabilis oxyfera*

OTV018
08:45
*S. ELLEUCHE, B. KLIPPEL, G. ANTRANIKIAN
Characterization of Novel Bacterial Alcohol Dehydrogenases Capable of Oxydizing 1,3-propanediol

OTV019
09:00
*S. HONDA, D. SCHEPS, L. KÜHNEL, B. NESTL, B. HAUER
Bacterial CYP153 monooxygenases as biocatalysts for the synthesis of ω-hydroxy fatty acids

OTV020
09:15
*S. SANÉ, S. RUBENWOLF, C. JOLIVALT, S. KERZENMACHER
Using yeast and fungi to produce electricity – Towards a self-regenerating enzymatic biofuel cell cathode

OTV021
09:30
*K. BÜHLER, R. KARANDE, B. HALAN, A. SCHMID
Biofilms – A new Chapter in Biocatalysis

OTV022
09:45
*S. SÖLLNER, M. RAHNERT, M. SIEMANN-HERZBERG, R. TAKORS, J. ALTENBUCHNER
Growth-decoupled, anaerobic succinate production from glycerol with pyruvate-kinase deficient *E. coli* mutants

OTV023
10:00
*J. VAN OOYEN, S. NOACK, M. BOTT, L. EGGELING
Gradual insight into *Corynebacterium glutamicum*'s central metabolism for the increase of L-lysine production

OTV024
10:15
H. WEINGART
Induction of systemic resistance in soybean by the antagonistic epiphyte *Pseudomonas syringae* 22d/93

MP II: Bacteria host cell interaction and host response

Lecture hall N5

Chair: Sven Hammerschmidt
Co-Chair: Christopher Weidenmaier

MPV009
08:30
*S. HANNEMANN, J.E. GALÁN
***Salmonella* Typhimurium Stimulated Transcriptional Response Aids Intracellular Replication**

MPV010
08:45
*M. FAULSTICH, J.-P. BÖTTCHER, T. MEYER, M. FRAUNHOLZ, T. RUDEL
Recruitment of PI3 kinase to caveolin 1 determines the switch from the extracellular to the disseminating stage of gonococcal infection

MPV011
09:00
*R. BÜCKER, J. BECKER, A.K. HEROVEN, P. DERSCH, C. WITTMANN
Systems biology of the pathogenic bacterium *Yersinia pseudotuberculosis*

MPV012
09:15
*A.D. ROEHRICH, E. GUILLOSSOU, R.B. SESSIONS, A.J. BLOCKER, I. MARTINEZ-ARGUDO
***Shigella* IpaD has a dual role in type III secretion system activation**

MPV013
09:30
*B. FRANZ, L. YUN-YUEH , M. TRUTTMANN, T. RIESS, M. FAUSTMANN, V. KEMPF, C. DEHIO
***Bartonella henselae* adhesin BadA negatively regulates effector secretion through the VirB/D4 type IV secretion system**

MPV014
09:45
*C. LASSEK, M. BURGHARTZ, D. CHAVES MORENO, B. HESSLING, A. OTTO, M. JAHN, D. BECHER, D. PIEPER, K. RIEDEL
A metaproteomic analysis of a human indwelling urinary catheter biofilm dominated by *Pseudomonas aeruginosa*

MPV015
10:00
*V. BEHRENDS, B. RYALL, J.E. ZLOSNIK, D.A. SPEERT, J.G. BUNDY, H.D. WILLIAMS
Metabolic adaptations of *Pseudomonas aeruginosa* during cystic fibrosis lung infections

MPV016
10:15
*S. VOSS, T. HALLSTRÖM, L. PETRUSCHKA, K. KLINGBEIL, K. RIESBECK, P. ZIPFEL, S. HAMMERSCHMIDT
Pneumococcal surface protein C: a multifunctional pneumococcal virulence factor and vitronectin-binding protein

Physiology I: Structural and regulatory aspects

Lecture hall N3

Chair: Gary Sawers
Co-Chair: Agnieszka Bera

PSV001
08:30
*L. KREUTER, S. DAXER, U. KÜPER, F. MAYER, V. MÜLLER, R. RACHEL, H. HUBER
The unusual cell architecture of *I. hospitalis* and consequences for its energy metabolism

PSV002
08:45
*S. BUBENDORFER, S. HELD, N. WINDEL, A. PAULICK, A. KLINGL, K. THORMANN
Function and specificity of the dual flagellar sytem in *Shewanella putrefaciens* CN-32

PSV003
09:00
*C. DOBERENZ, L. BEYER, D. FALKE, M. ZORN, B. THIEMER, G. SAWERS
Pyruvate formate-lyase Controls Formate Translocation by the FocA Channel

PSV004
09:15
*A. SCHNORPFEIL, M. MÜLLER, R. BRÜCKNER
The small non-coding csRNAs controlled by the response regulator CiaR affect β-lactam sensitivity and competence in *Streptococcus pneumoniae*

Tuesday, March 20, 08:30–10:30

PSV005
09:30
*D. VASILEVA, H. JANSSEN, H. BAHL
Fur mediates control of riboflavin biosynthesis, iron uptake and energy metabolism in *Clostridium acetobutylicum*

PSV006
09:45
*A. UHDE, T. MAEDA, L. CLERMONT, J.-W. YOUN, V.F. WENDISCH, R. KRÄMER, K. MARIN, G.M. SEIBOLD
Improved Glucosamine Utilization by *Corynebacterium glutamicum* and its application for L-Lysine production

PSV007
10:00
*P. PETERS-WENDISCH, K.C. STANSEN, S. GÖTKER, V.F. WENDISCH
Characterization of biotin protein ligase from *Corynebacterium glutamicum*: enzymatic analysis, physiological role and biotechnological application

PSV008
10:15
*D. LEHMANN, T. LÜTKE-EVERSLOH
Physiological effects of disrupting the acetate and acetone formation pathways in *Clostridium acetobutylicum*

Metabolic regulation and signaling II: Responses and pathways

Lecture hall N2

Chair: Gottfried Unden
Co-Chair: Michael Bott

RSV009
08:30
*F. COMMICHAU, S. THOLEN, K. GUNKA
A high-frequency mutation in *Bacillus subtilis*: Requirements for the decryptification of the *gudB* glutamate dehydrogenase gene

RSV010
08:45
*A. CARIUS, L. CARIUS, H. GRAMMEL
The PpsR Protein in *Rhodospirillum rubrum*: A major metabolism coordinator

RSV011
09:00
*B. BERGHOFF, Y. HERMANNS, G. KLUG
Fine-tuning of sulfur metabolism by a peptide-coding sRNA in the photooxidative stress response of *Rhodobacter*

RSV012
09:15
*M. VOCKENHUBER, B. SUESS
The Conserved sRNA scr5239 Controls DagA Expression by Translational Repression

RSV013
09:30
*A. SCHRAMM, B. LEE, T. JEGANATHAN, P.I. HIGGS
Two hybrid histidine kinases utilize inter- and intra-protein phosphorylation to regulate developmental progression in *Myxococcus xanthus*

RSV014
09:45
*S. SCHWEIKERT, S. BRINGER, M. BOTT
Studies of an Fnr-like transcriptional regulator in *Gluconobacter oxydans* 621H

RSV015
10:00
*D. LÜTTMANN, B. GÖRKE
EIIA^{Ntr} of the nitrogen phosphotransferase system regulates expression of the *pho* regulon via interaction with histidine kinase PhoR in *Escherichia coli*

RSV016
10:15
*C. MÜLLER, S. FETZNER
A *Pseudomonas putida* bioreporter strain for the detection of alkylquinolone-converting enzymes

Soil microbiology II: Geomicrobiology and environmental microbiology

Lecture hall N4

Chair: Andreas Kappler

SMV009
08:30
*A. BAHR, P. BOMBACH, A. FISCHER
Evidence of aerobic polycyclic aromatic hydrocarbon (PAH) biodegradation in a contaminated aquifer by combining BACTRAP®s and laboratory microcosms

SMV010
08:45
M.B. LOGANATHAN, A. KAPPLER, *S. BEHRENS
Cobalt trace metal requirement for reductive dechlorination of trichloroethene by Dehalococcoides

SMV011
09:00
*M. SCHMIDT, I. NIJENHUIS, D. WOLFRAM, S. DEVAKOTA, J. BIRKIGT, B. KLEIN, H.H. RICHNOW
Anaerobic transformation of chlorobenzene and dichlorobenzene in highly contaminated groundwater

SMV012
09:15
*S. ZIEGLER, K. DOLCH, J. MAJZLAN, J. GESCHER
On the distinct physiological capabilities of so far uncultured archaea in acidophilic biofilms

SMV013
09:30
*S. JECHALKE, C. KOPMANN, I. ROSENDAHL, J. GROONEWEG, E. KRÖGERRECKLENFORT, U. ZIMMERLING, V. WEICHELT, G.-C. DING, J. SIEMENS, W. AMELUNG, H. HEUER, K. SMALLA
Effects of sulfadiazine entering via manure into soil on abundance and transferability of antibiotic resistance in the rhizosphere of grass and maize

SMV014
09:45
*M. PESTER, B. HAUSMANN, N. BITTNER, P. DEEVONG, M. WAGNER, A. LOY
The 'rare biosphere' contributes to wetland sulfate reduction – fameless actors in carbon cycling and climate change

SMV015
10:00
*C. SCHMIDT, E.-D. MELTON, A. KAPPLER
Microbial iron cycling in freshwater sediments

SMV016
10:15
*D. KANAPARTHI, M. DUMONT, B. POMMERENKE, P. CASPER
Autotrophic Fe(II) oxidizing bacteria in the littoral sediment of Lake Große Fuchskuhle

Tuesday, March 20, 08:30–10:30

MP III: Virulence factors, function and regulation

Lecture hall N9

Chair: Thomas Rudel
Co-Chair: Samuel Wagner

MPV017
08:30
I. AHMAD, A. LAMPROKOSTOPOULOU, S. LE GUYON, E. STRECK, M. BARTHEL, V. PETERS, W.-D. HARDT, *U. RÖMLING
Complex c-di-GMP signaling networks mediate the transition between biofilm formation and virulence properties in *Salmonella enterica* serovar Typhimurium

MPV018
08:45
*M. BURGHARTZ, P. TIELEN, R. NEUBAUER, D. JAHN, M. JAHN
Characterization of bacterial strains isolated from community acquired asymptomatic catheter associated urinary tract infections

MPV019
09:00
*B. WALDMANN
Global discovery of virulence-associated small RNAs in *Yersinia pseudotuberculosis*

MPV020
09:15
*V. SCHÜNEMANN, K. BOS, H. POINAR, J. KRAUSE
Fishing for ancient pathogens: A draft genome of a *Yersinia pestis* strain from the medieval Black Death

MPV021
09:30
*T. JAEGER, J.G. MALONE, P. MANFREDI, A. DÖTSCH, A. BLANKA, S. HÄUSSLER, U. JENAL
The YfiBNR signal transduction mechanism reveals novel targets for the evolution of persistent *Pseudomonas aeruginosa* in cystic fibrosis airways

MPV022
09:45
*L. DENKEL, S. HORST, S. FAZLE ROUF, V. KITOWSKI, O. BÖHM, M. RHEN, T. JÄGER, F.-C. BANGE
Methionine sulfoxide reductases defend *Salmonella* Typhimurium from oxidative stress and provide bacterial pathogenesis

MPV023
10:00
*G. MARINCOLA, T. SCHÄFER, K. OHLSEN, C. GOERKE, C. WOLZ
RNase Y of *Staphylococcus aureus* does not result in bulk mRNA decay but in activation of virulence genes

Cell envelope I: Membrane transport and dynamics

Lecture hall N1

Chair: Jan Tommassen
Co-Chair: Doron Rapaport

CEV001
08:30
*H. STRAHL, L. HAMOEN
The bacterial MreB cytoskeleton organizes the cell membrane

CEV002
08:45
*D. WOLF, P. DOMÍNGUEZ-CUEVAS, R. DANIEL, T. MASCHER
Cell envelope stress response in cell wall-deficient L-forms of *Bacillus subtilis*

CEV003
09:00
*S. HEBECKER, W. ARENDT, T. HASENKAMPF, I. HEINEMANN, D. SÖLL, D. JAHN, J. MOSER
Mechanism of substrate recognition of the tRNA-dependent alanyl-phosphatidylglycerol synthase from *Pseudomonas aeruginosa*

CEV004
09:15
T. BAUMGARTEN, S. STEFANIE SPERLING, J. SEIFERT, F. STEINIGER, J.A. MÜLLER, L.Y. WICK, *H.J. HEIPIEPER
Membrane vesicle formation in *Pseudomonas putida* DOT-T1E as multiple stress response mechanism enhances cell surface hydrophobicity and biofilm formation

CEV005
09:30
*I. MALDENER, P. STARON, K. FORCHHAMMER
A Novel ATP-Driven Pathway of Glycolipid Export for Cell Envelope Formation Involving TolC

CEV006
09:45
A. EDWARDS, J.A. DOWNIE, *M. KREHENBRINK
A non-classical periplasmic protein targeting mechanism

CEV007
10:00
*J. LEO, P. OBERHETTINGER, M. SCHÜTZ, M. FLÖTENMEYER, I. AUTENRIETH, D. LINKE
Structural and functional dissection of the Invasin-Intimin family of bacterial adhesins

CEV008
10:15
*M. WENZEL, A.I. CHIRIAC, B. ALBADA, A. OTTO, A. KNÜFER, D. BECHER, L. HAMOEN, H.-G. SAHL, N. METZLER-NOLTE, J.E. BANDOW
The Cell Envelope as Target of a Novel Antimicrobial Peptide

Wednesday, March 21, 09:00–11:00

Open Topics IV: Environmental Microbiology

Lecture hall N7

Chair: Michael Schloter
Co-Chair: Barbara Morasch

OTV025
09:00
*J. DEUTZMANN, B. SCHINK
Anaerobic denitrifying methane oxidation in a deep oligotrophic freshwater lake

OTV026
09:15
*F. SCHULZ, M. KUROLL, K. AISTLEITNER, M. HORN
Life inside the Nucleus – An Unusual Symbiont of Amoebae Related to Rickettsiae

OTV027
09:30
*A. QUAISER, X. BODI, A. DUFRESNE, A. DHEILLY, S. COUDOUEL, D. NAQUIN, A. FRANCEZ, P. VANDENKOORNHUYSE
Functional community analysis of a microbial mat involved in the oxydation of iron by metatranscriptomics

OTV028
09:45
G. PILLONI, M. GRANITSIOTIS, *T. LUEDERS
Testing the limits of 454 pyrotag sequencing: reproducibility and quantitative assessment

OTV029
10:00
*S.E. RUFF, J. BIDDLE, A. TESKE, A. RAMETTE, K. KNITTEL, A. BOETIUS
Microbial Communities of Marine Methane Seeps: Sketching the Big Picture

OTV030
10:15
*M. BLÖTHE, A. SCHIPPERS
Prokaryotic diversity in Pacific Ocean manganese nodules

OTV031
10:30
S. GLAESER, F. LEUNERT, I. SALKA, H.-P. GROSSART, *J. GLAESER
Inhibition of heterotrophic bacteria by solar radiation in a humic lake

OTV032
10:45
*X. DONG, M. ENGEL, M. SCHLOTER
Phylogenetic characterization and comparison of microbial communities in mesophilic and thermophilic anaerobic digesters

MP IV: Microbial Pathogenicity/Human Microbiota

Lecture hall N5

Chair: Hubert Hilbi
Co-Chair: Julia-Stefanie Frick

MPV024
09:00
M. CHRISTNER, M. BUSCH, C. HEINZE, M. KOTASINSKA, G. FRANKE, *H. ROHDE
***sarA* negatively regulates *Staphylococcus epidermidis* biofilm formation by modulating expression of 1 MDa extracellular matrix binding protein and autolysis dependent release of eDNA**

MPV025
09:15
*A. KOENIGS, P. KRAICZY, C. SIEGEL, S. FRÜH, T. HALLSTRÖM, C. SKERKA, P.F. ZIPFEL
CspA of *Borrelia burgdorferi* is a regulator of the alternative pathway

MPV026
09:30
S. WANNER, M. RAUTENBERG, S. BAUR, L. KULL, *C. WEIDENMAIER
Zwitterionic cell wall polymers of bacterial pathogens-important modulators of T cell dependent infections

MPV027
09:45
*M.M. HEIMESAAT, A. FISCHER, R. PLICKERT, L.-M. HAAG, B. OTTO, A.A. KÜHL, J.I. DASHTI, A.E. ZAUTNER, M. MUNOZ, C. LODDENKEMPER, U. GROß, U.B. GÖBEL, S. BERESWILL
Novel Murine Infection Models Provide Deep Insights into the „Ménage à Trois" of *Campylobacter jejuni*, Microbiota and Host Innate Immunity

HMV001
10:00
*A. BRAUNE, M. BLAUT
Deglycosylation of polyphenolic *C*-glucosides by a human gut bacterium

HMV002
10:15
*S. KROHN, J. HARTMANN, A. BRODZINSKI, A. CHATZINOTAS, S. BÖHM, T. BERG
Application of real-time PCR, T-RFLP and direct sequencing for the identification of polybacterial 16S rRNA genes in ascites

HMV003
10:30
*K. FÖRSTER-FROMME, S. MITRA, T. SCHEURENBRAND, S. BISKUP, D. BOEHM, D.H. HUSON, S.C. BISCHOFF
Analysis of the intestinal microbiota using SOLiD 16SrRNA gene sequencing and SOLiD shotgun sequencing

HMV004
10:45
*D. JANEK, B. KRISMER, A. PESCHEL
Bacteriocin production of staphylococcal nasal isolates

Physiology II: Redox systems and cytochromes

Lecture hall N3

Chair: Reinhold Brückner
Co-Chair: Klaus Hantke

PSV009
09:00
*J. ZHANG, P. FRIEDRICH, B.M. MARTINS, W. BUCKEL
4-Hydroxybutyryl-CoA dehydratase, a radical enzyme in metabolic pathways of anaerobic Bacteria and Archaea

PSV010
09:15
*J. DERMER, G. FUCHS
Characterization of a novel anaerobic steroid C25 dehydrogenase (DMSO reductase family) in *Sterolibacterium denitrificans*

PSV011
09:30
*R. ROSENTHAL, T.J. ERB
Investigating the reaction mechanism of crotonyl-CoA carboxylase/reductase: Exploring the bio(techno)logical potential of reductive carboxylation

PSV012
09:45
*M. KERN, J. SIMON, M.G. KLOTZ
The unconventional octahaem cytochrome *c* MccA is the terminal reductase of *Wolinella succinogenes* sulfite respiration

Wednesday, March 21, 09:00–11:00

PSV013
10:00
*M. SCHLEBUSCH, W. HAUF, K. FORCHHAMMER
Localization and regulation of PHB granules in *Synechocystis sp.* PCC 6803

PSV014
10:15
*J. MOSER, C. LANGE, M. BRÖCKER, M. SAGGU, F. LENDZIAN, H. SCHEER, D. JAHN
Biosynthesis of (Bacterio)chlorophylls: ATP-Dependent Transient Subunit Interaction and Electron Transfer of Dark-operative Protochlorophyllide Oxidoreductase

PSV015
10:30
*M. JETTEN, M. SMEULDERS, H. OP DEN CAMP, T. BARENDS, I. SCHLICHTING
Carbon disulfide hydrolase: a new enzyme for CS_2 conversion in acidothermophilic microorganisms

PSV016
10:45
*K. SCHLEGEL, V. LEONE, J. FARALDO-GÓMEZ, V. MÜLLER
A promiscuous archaeal ATP synthase concurrently coupled to Na^+ and H^+ translocation

Secondary Metabolites II: Fermentation Studies/ Microbial Survival Strategies II

Lecture hall N2

Chair: Lutz Heide, Christiane Wolz
Co-Chair: Shu-Ming Li, Knut Ohlsen

MEV009
09:00
*Z.-E. BILAL, A. YOUSAF
Biosynthesis of Cephlaosporin C Through Improved strains of *Aspergillus* and *Acremonium species*

MEV010
09:15
*A. KLINGNER, A. BARTSCH, J. BECKER, C. WITTMANN
Systems biology of the marine antibiotic producer *Phaeobacter gallaeciensis*

MEV011
09:30
*A.R. WEIZ, K. ISHIDA, K. MAKOWER, N. ZIEMERT, C. HERTWECK, E. DITTMANN
Characterization and manipulation of the biosynthetic pathway of cyanobacterial tricyclic microviridins in *E. coli*

MEV012
09:45
*A. JONES, S. OTTILIE, A. EUSTÁQUIO, D. EDWARDS, L. GERWICK, B. MOORE, W. GERWICK
Evaluation of *Streptomyces coelicolor* as a heterologous expression host for natural products from marine filamentous cyanobacteria

SSV009
10:00
S. SCHUSTER
Mathematical modelling of cooperation and cheating in survival strategies of microorganisms

SSV010
10:15
*R. BERTRAM, K. LEWIS, S. LECHNER
***Staphylococcus aureus* persister cells tolerant to bactericidal antibiotics**

SSV011
10:30
*N. STREMPEL, M. NUSSER, G. BRENNER-WEISS, J. OVERHAGE
Sodium hypochlorite stimulates c-di-GMP synthesis and biofilm formation in *Pseudomonas aeruginosa*

SSV012
10:45
B. COLLEY, S. KJELLEBERG, *J. KLEBENSBERGER
SiaABCD, a signaling pathway controlling autoaggregation in *Pseudomonas aeruginosa*

Bacterial Differentiation: New aspects of bacterial cell differentiation

Lecture hall N4

Chair: Martin Thanbichler
Co-Chair: Günther Muth

BDV001
09:00
*K. JONAS, M.T. LAUB
Setting the pace: Mechanisms controlling the temporal regulation of the *Caulobacter crescentus* cell cycle

BDV002
09:15
*D. KIEKEBUSCH, K. MICHIE, L.-O. ESSEN, J. LÖWE, M. THANBICHLER
How to generate a protein gradient within a bacterial cell: dynamic localization cycle of the cell division regulator MipZ

BDV003
09:30
*C. KAIMER, D. ZUSMAN
Regulation of cellular reversals in *Myxococcus xanthus*

BDV004
09:45
*J. LEHNER, Y. ZHANG, S. BERENDT, I. MALDENER, K. FORCHHAMMER
The cell wall amidase AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme* ATCC 29133

BDV005
10:00
*F.D. MÜLLER, O. RASCHDORF, E. KATZMANN, M. MESSERER, D. SCHÜLER
Functional analysis of cytoskeletal proteins implicated in magnetosome formation and cell division in *Magnetospirillum gryphiswaldense*

BDV006
10:15
*A. TREUNER-LANGE, A. HARMS, L. SOGAARD-ANDERSEN
The PomX protein is required for cell division in *Myxococcus xanthus*

BDV007
10:30
*J.C. GARCIA-BETANCUR, A. YEPES GARCIA, D. LOPEZ
Cell Differentiation in Biofilms Communities of *Staphylococcus aureus*

Wednesday, March 21, 09:00–11:00

BDV008
10:45
*C. JOGLER, F.O. GLÖCKNER, R. KOLTER
Eating and being eaten: What bacterial cell biology can tell us about eukaryogenesis

Cell Envelope II: Cell wall synthesis and maintenance

Lecture hall N9

Chair: Hans-Georg Sahl
Co-Chair: Christoph Mayer

CEV009
09:00
*J. DOMINGUEZ-ESCOBAR, A. CHASTANET, A.H. CREVENNA, R. WEDLICH-SÖLDNER, R. CARBALLIDO-LÓPEZ
Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria

CEV010
09:15
*J. GISIN, A. SCHNEIDER, B. NÄGELE, C. MAYER
A shortcut pathway to UDP-MurNAc through peptidoglycan recycling in *Pseudomonas*

CEV011
09:30
*D. MÜNCH, T. ROEMER, S.H. LEE, M. ENGESER, H.-G. SAHL , T. SCHNEIDER
Identification and *in vitro* analysis of the GatD/MurT enzyme-complex catalyzing lipid II amidation in *S. aureus*

CEV012
09:45
*B. SIEGER, M. BRAMKAMP
Synthetic analysis of the apical cell wall synthesis machinery from *Corynebacterium glutamicum*

CEV013
10:00
*T. KOHLER, N. GISCH, M. SCHLAG, K. DARM, U. VÖLKER, U. ZÄHRINGER, S. HAMMERSCHMIDT
Repeating structures of different Gram-positive surface-proteins are essential for the bacterial interaction with human Thrombospondin-1

CEV014
10:15
*M. SCHLAG, S. ZOLL, A. SHKUMATOV, M. RAUTENBERG, T. STEHLE, F. GÖTZ
The structural basis of staphylococcal cell wall recognition by SH3b domains

CEV015
10:30
A. HENRICH, J.B. SCHULTE, A.W. ECK, *G.M. SEIBOLD
Identification of the trehalose uptake system TusEFGK$_2$ of *Corynebacterium gluctamicum* and characterization of its role in the biosynthesis of mycolic acids

CEV016
10:45
*B. MEYER, B. ZOLGHADR, E. PEYFOON, M. PABST, M. PANICO, H.R. MORRIS, P. MESSNER, C. SCHÄFFER, A. DELL, S.-V. ALBERS
Elucidation of the *N*-glycosylation pathway in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*

Fungi: Fungal genetics and physiology

Lecture hall N1

Chair: Reinhard Fischer
Co-Chair: Rüdiger Hampp

FUV001
09:00
*M. NOWROUSIAN, I. TEICHERT, G. WOLFF, U. KÜCK
Genomics and transcriptomics based on next-generation sequencing techniques to characterize fungal developmental genes

FUV002
09:15
*J. RÖHRIG, R. FISCHER
VipA – a novel player in light sensing and development in *Aspergillus nidulans*

FUV003
09:30
*K. GRÜTZMANN, K. SZAFRANSKI, M. POHL, K. VOIGT, A. PETZOLD, S. SCHUSTER
Alternative splicing in the fungal kingdom

FUV004
09:45
*A. YEMELIN, S. MATHEIS, E. THINES, K. ANDRESEN, A.J. FOSTER
Transcription factors controlling sporulation in *Magnaporthe oryzae*

FUV005
10:00
*S. BRAUS-STROMEYER, V.T. TRAN, C. TIMPNER, C. HOPPENAU, S. SINGH, A. KÜHN, H. KUSCH, O. VALERIUS, G. BRAUS
The interaction of the plant-pathogen *Verticillium longosporum* and its host *Brassica napus* and insights into the evolutionary origin of the fungal hybrid

FUV006
10:15
*R. REINA , C. LIERS, R. ULLRICH, I. GARCIA-ROMERA, M. HOFRICHTER, E. ARANDA
Induction of manganese peroxidases of wood and leaf-litter colonizing agaricomycetes by olive oil mill residues

FUV007
10:30
*N. HORLACHER, S. SCHREY, J. NACHTIGALL, R. HAMPP, R. SÜSSMUTH, H.-P. FIEDLER
The plant pathogenic fungus *Heterobasidion* produces plant hormone-like compounds to elude the plant defense

FUV008
10:45
*T. WOLLENBERG, J. DONNER, K. ZUTHER, L. STANNEK, J. SCHIRAWSKI
Discovering host specificity candidate genes of *Sporisorium reilianum* by genotyping mixed-variety offspring

ISV01

Die verborgene Welt der Bakterien und ihre Bedeutung für das Leben auf der Erde

K.-H. Schleifer
Technische Universität München, Mikrobiologie, München, Germany

Bei Bakterien denken die meisten Menschen an Krankheitserreger. Doch die überwiegende Mehrheit dieser Organismen ist harmlos oder sogar nützlich. Sie spielen eine wichtige Rolle bei der Herstellung fermentierter Lebensmittel oder in der weißen Biotechnologie. Die zellkernlosen Prokaryoten (Bakterien + Archaeen) sind jedoch noch aus anderen Gründen sehr wichtig. Sie kommen in ungeheuer großen Zahlen vor und machen ca. 50% der globalen Biomasse aus. Leider ist bisher nur ein Bruchteil von ihnen bekannt, da sie als Reinkultur nicht zugänglich sind. Durch genotypische Methoden, insbesondere durch vergleichende Sequenzanalyse der 16S-rRNS Gene ist es allerdings möglich, die Organismen auch ohne vorherige Kultivierung zu identifizieren. Mit Hilfe maßgeschneiderter, fluoreszenzmarkierter Oligonukleotidsonden, die an komplementäre Sequenzen der 16S-rRNS binden, lassen sich die Organismen auch *in situ* nachweisen und identifizieren. Dies soll anhand verschiedener Beispiele belegt werden.
Die Prokaryoten sind die Wegbereiter der Biosphäre. Für mindestens 2 Milliarden Jahre waren sie die einzigen Lebewesen auf unserem Planeten. Sie waren an der Entstehung der höheren Lebewesen (Eukaryoten) beteiligt, und die Cyanobakterien sorgten für den nötigen Sauerstoff auf der Erde. Der Nährstoffkreislauf, insbesondere Stickstoff-und Schwefelkreislauf, wäre ohne die Prokaryoten unvollständig. Überdies zeichnen sie sich durch einzigartige Mechanismen der Energiegewinnung aus, und sie setzen auch die Grenzen des Lebens fest. Sie wachsen überall, wo noch flüssiges Wassers vorkommt.
Die Bakterien spielen auch eine besondere Rolle in der Evolution und Ökologie der Eukaryoten. Sie können als Kommensalen, Endo- oder Ektosymbionten vorkommen. Dies soll an verschiedenen Beispielen gezeigt werden.
Bakterien und Archaeen sind durch ihre vielfältigen Aktivitäten wichtig für Umwelt und Klima. Sie sind entscheidend an Aufbau und Erhalt der Biosphäre beteiligt. Ohne sie wäre die Mineralisierung organischer Stoffe unvollständig und ohne sie gäbe es auch nicht die typischen Eukaryoten. Der Vorläufer der heutigen Mitochondrien, den Energiekraftwerken der eukaryotischen Zellen, gehört zu den Alpha-Proteobakterien und die Chloroplasten, in denen die Photosynthese stattfindet, stammen von Cyanobakterien ab. All dies spricht dafür, dass es ohne Bakterien kein Leben auf unserem Planeten gäbe.

ISV02

From microorganisms to the atmosphere: flooded soils and the methane cycle

R. Conrad
Max-Planck-Institut für terrestrische Mikrobiologie, Biogeochemie, Marburg, Germany

Flooded soils such as rice fields and wetlands are the most important source for the greenhouse gas methane. Rice fields, in particular, serve as model for studying the role of the structure of anaerobic microbial communities for ecosystem functioning and the partitioning of carbon flux along different paths of degradation of organic matter to methane. Flooded soils are relatively rapidly depleted of oxygen and other oxidants such as ferric iron and sulfate. Then, organic matter degradation results in the production methane. Methane is eventually produced from different types of organic matter, mainly from plant litter, root exudates, and soil organic matter. Methane production is achieved by a community consisting of hydrolytic, fermenting and methanogenic microorganisms. Acetate and hydrogen (plus CO_2) are the two most important fermentation products that are used as methanogenic substrates to different extent. The transport of CH_4 to the atmosphere is mainly partitioned between transport through the aerenchyma system of plants, gas ebullition and diffusion. Transport through oxygenated zones such as the surface soil or the rhizosphere results in oxidation of a substantial percentage of methane by methanotrophic bacteria thus attenuating the methane flux into the atmosphere. Tracer experiments (e.g. using stable carbon isotopes) are useful for quantifying the partitioning of carbon flux along different paths and for elucidating the active microbial groups involved in carbon transformation.

ISV03

Physiology, mechanisms and habitats of microbial Fe(II) oxidation

A. Kappler
University of Tübingen, Geomicrobiology, Center for Applied Geosciences, Tübingen, Germany

The two most important redox states of iron in the environment are Fe(II) [ferrous iron] and Fe(III) [ferric iron]. Dissolved Fe(II), relatively soluble Fe(II) minerals and poorly soluble Fe(III) minerals are abundant in pH-neutral soils and sediments. Redox transformation of iron leading either to dissolution, transformation or precipitation of iron minerals is used by many microorganisms to produce energy and to grow. Oxidation of dissolved ferrous iron [Fe(II)] at neutral pH can be catalyzed by acidophilic aerobic and neutrophilic microaerophilic, nitrate-reducing and even phototrophic microorganisms. This contribution will present the current knowledge and new results regarding mechanisms, physiology, ecology and environmental implications of microbial Fe(II) oxidation. Special focus will be on microaerophilic Fe(II)-oxidizing bacteria that thrive in gradients of ferrous iron and oxygen (e.g. at the surface of rice roots in paddy soil), phototrophic Fe(II)-oxidizing autotrophs living in surface near environments such as littoral sediments, and finally on nitrate-reducing bacteria oxidizing Fe(II) in soils and sediments.

ISV04

Assembly and function of archaeal surface structures

S.-V. Albers
Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany

Archaea, the third domain of life, possess a variety of surface structures such as pili and flagella. These structures have in common that they are composed of subunits that are found in bacterial type IV pili which among others are involved in bacterial pathogenesis. The archaeal pili and flagella systems appear to be much simpler than their bacterial counterparts and are therefore well suited model systems to understand the mechanistic of the assembly process.The thermoacidophilic archaeon Sulfolobus acidocaldarius exhibits three different surface appendages, (i) flagella, (ii) thin pili, and (iii) UV light induced pili. In Sulfolobus the flagellum is mainly involved in adhesion and surface motility, which seems to be inhibited by the thin pili. The UV induced pili initiate cell aggregation after DNA damage and subsequent DNA repair by conjugation. Next to the physiological function of these surface structures our understanding of their assembly will be discussed.

ISV05

Current views on the role as well as the fate of host cells during infection

Thomas F. Meyer and coworkers
Department of Molecular Biology, Max Planck Institute for Infection Biology, Berlin, Germany

Infectious disease research has led us to the realization that the initiation and progression of infection are critically dependent on both pathogen and host determinants. Microbial virulence factors have been studied in great detail over the past decades; however, the role of host determinants as the counterparts of pathogen virulence factors and signal transduction elements has been less intensely pursued. With the discovery of RNAi, an extremely useful tool has become available that facilitates the assessment of host-cell determinants and their role in infection at the genome-wide level. Here, I present two examples of global host-cell function analysis, addressing influenza virus and *Chlamydia* infections (1,2), and discuss the implications for the development of a novel class of therapeutic drugs as well as for our future understanding of host susceptibility to infection and morbidity/mortality determinants.
Host cells are not merely vehicles for pathogen replication; it appears host cells are also subject to genetic and epigenetic modifications during infection, and are therefore capable of acquiring heritable features that may underlie pathological sequelae, including cancer. The gastric pathogen *Helicobacter pylori* is the paradigm of a cancer-inducing bacterium (3). We, and others, can show that *H. pylori* and other bacterial pathogens are capable of causing genetic and epigenetic lesions in infected cells (4). However, DNA damage alone does not seem to be sufficient in itself for carcinogenesis. Other features such as persistence of infection and mitogenic stimuli are likely cofactors (5).

1. Karlas, A., N.Machuy, Y.Shin, K.-P.Pleissner, A.Artarini, D.Heuer, D.Becker, H.Khalil, L.A.Ogilvie, S.Hess, A.P.Mäurer, E.Müller, T.Wolff, T.Rudel, and T.F.Meyer. 2010. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. Nature 463:818-822.
2. Gurumurthy, R.K., A.P.Mäurer, N.Machuy, S.Hess, K.P.Pleissner, J.Schuchhardt, T.Rudel, and T.F.Meyer. 2010. A loss-of-function screen reveals Ras- and Raf-independent MEK-ERK signaling during Chlamydia trachomatis infection. Science Signaling 3:ra21.
3. Bauer, B., and T.F.Meyer. 2011. The human gastric pathogen Helicobacter pylori and its association with gastric cancer and ulcer disease. Ulcers. doi:10.1155/2011/340157
4. Fassi Fehri, L., C.Rechner, S.Janssen, T.N.Mak, C.Holland, S.Bartfeld, H.Bruggemann, and T.F.Meyer. 2009. Helicobacter pylori-induced modification of the histone H3 phosphorylation status in gastric epithelial cells reflects its impact on cell cycle regulation. Epigenetics. 4:577-586.
5. Kessler, M., J.Zielecki, O.Thieck, H.J.Mollenkopf, C.Fotopoulou, and T.F.Meyer. 2011. Chlamydia Trachomatis Disturbs Epithelial Tissue Homeostasis in Fallopian Tubes via Paracrine Wnt Signaling. Am. J Pathol..180:186-198.

ISV06

No abstract submitted!

ISV07
Teichoic acids in Gram-positive cell wall function and host interaction

A. Peschel
Universitätsklinikum Tübingen, Medical Microbiology and Hygiene Department, Tübingen, Germany

The presence of teichoic acids or related polyanionic glycopolymers has remained an enigmatic trait of most Gram-positive bacterial cell envelopes. Recent advances in teichoic acids biosynthesis and the availability of defined mutants now permit to explore the roles of teichoic acids, which exhibit extensive species or strain-specific differences in composition and glycosylation but share polyanionic properties. Although not essential to bacterial viability recent studies indicate that teichoic acid are crucial for directing cell envelope metabolism and turn-over and for governing the capacity of host-adapted Gram-positive bacteria to colonize and infect mammalian host organisms. Therefore, teichoic acids represent attractive targets for new antibacterial therapeutics against staphylococci and other Gram-positive human pathogens.

ISV08
Out of the iron age: the battle for zinc

J. Tommassen
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The cell envelope of Gram-negative bacteria consists of two membranes, which are separated by the periplasm containing the peptidoglycan layer. The outer membrane is an asymmetrical bilayer consisting of phospholipids in the inner leaflet and lipopolysaccharides in the outer leaflet. It functions as a barrier for harmful compounds from the environment including many antibiotics. In contrast to the inner membrane, the outer membrane is not energized by a proton gradient and ATP is not available in the periplasm. The lack of direct energy sources may complicate transport processes across the outer membrane. Nevertheless, nutrients are taken up from the environment and proteins, including toxins and hydrolytic enzymes, are secreted.
Most nutrients pass the outer membrane by passive diffusion via outer membrane proteins, collectively called porins, which form large open channels in the outer membrane. Hence, in this case, energy availability is not an issue. However, diffusion is an option only when the extracellular concentration of the solute is high. The uptake of nutrients that are too dilute in the environment or whose size exceeds the exclusion limit of the porins is dependent on specific receptors and requires energy. The energy source utilized is the proton-motive force across the inner membrane, which is coupled to the transport process in the outer membrane via a complex of three proteins, the TonB complex. The receptors involved are called TonB-dependent family (Tdf) members.
In the vertebrate host, iron is sequestered by the iron-transport and -storage proteins transferrin and lactoferrin. Hence, the concentration of free iron is extremely low and restricts microbial growth, a mechanism known as nutritional immunity. Many Gram-negative pathogens respond to iron limitation by the production and secretion of small iron-chelating compound, called siderophores, which bind ferric ions with very high affinity. In addition, they produce Tdf receptors for the uptake of ferric-siderophore complexes. Other pathogens, including *Neisseria meningitidis*, do not produce siderophores, but they produce receptors for the iron-binding proteins of the host.
Since efficient iron acquisition is an important virulence factor, it has been studied extensively in many pathogens. However, nutritional immunity in the host is not restricted to iron limitation. Also other metals, including zinc, manganese and nickel, can be limiting in the host, which responds to infections by the production of metal-binding proteins, such as metallothioneins and calprotectin. How these metals are transported across the outer membrane is largely unknown.
N. meningitidis normally lives as a commensal in the upper respiratory tract of up to 20% of the population but occasionally causes sepsis an meningitis. A broadly cross-protective vaccine is not available. The presence of 12 Tdf receptors has been identified by analyzing the available genome sequences. Five of these receptors have well-defined roles in iron acquisition and their expression is induced under iron limitation. Microarray studies revealed that the expression of several other Tdf receptors is unresponsive to iron availability; hence, we considered the possibility that these receptors are involved in the uptake of other metals. We have studied the response of *N. meningitidis* to zinc limitation and found that the expression of two Tdf receptors is specifically induced under those conditions. We have demonstrated that these receptors are involved in zinc acquisition and identified their ligands. The results demonstrate how *N. meningitidis* evades nutritional immunity imposed by the metal-binding proteins of the host. The receptors involved are attractive vaccine candidates.

ISV09
Origins and proliferation of L-form (cell-wall deficient) *Bacillus subtilis*

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The cell wall is a defining structure of bacterial cells. It provides a protective outer shell and is crucial in pathogenesis as well as the target for important antibiotics. Synthesis of the wall is organised by cytoskeletal proteins homologous to tubulin (FtsZ) and actin (MreB). Because all major branches of the bacterial lineage possess both wall and cytoskeleton, these were probably present in the last common ancestor of the bacteria. L-forms are unusual variants of bacteria that lack the wall and are found in various specialised habitats, possibly responsible for a range of chronic and persistent diseases. We have developed a model system for studying the L-form state in *Bacillus subtilis* (Leaver et al., 2009, *Nature* 457, 849-53). Molecular genetic analysis has revealed a number of discrete steps required for the transition from the walled to the non-walled state (Domínguez-Cuevas et al., 2012, *Mol Microbiol* 83, 52-66). Unexpectedly, it has also shown that proliferation of L-forms is completely independent of the normally essential FtsZ or MreB cytoskeletal systems and occurs by a membrane blebbing or tubulation process. Genetic analysis has identified factors required for proliferation of L-forms, and so far these results point to membrane dynamics as being of critical importance. L-forms may provide an interesting model for considering how primitive cells proliferated before the invention of the cell wall.

ISV10
Positive regulation of cell division site positioning in bacteria by a ParA protein

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In all cells, accurate positioning of the division site is essential for generating appropriately-sized daughter cells with a correct chromosome number. In bacteria, cell division generally occurs at midcell and initiates with assembly of the tubulin homologue FtsZ into a circumferential ring-like structure, the Z-ring, underneath the cell membrane at the incipient division site. Subsequently, FtsZ recruits the remaining components of the division machinery needed to carry out cytokinesis. Thus, the position of Z-ring formation dictates the division site. Consistently, all known systems that regulate positioning of the division site control Z-ring positioning. These systems act as negative regulators to inhibit Z-ring formation at the cell poles and over the nucleoid, leaving only midcell free for Z-ring formation. Here we show that the ParA homologue PomZ positively regulates Z-ring positioning in *Myxococcus xanthus*. Lack of PomZ results in division defects, a reduction in Z-ring formation, and abnormal positioning of the few Z-rings formed. PomZ localization is cell cycle regulated and culminates at midcell before and independently of FtsZ suggesting that PomZ recruits FtsZ to midcell. FtsZ alone does not polymerize, however, FtsZ polymerization is directly stimulated by PomZ in vitro. Thus, PomZ positively regulates positioning of the division site by recruiting FtsZ and providing positional information for Z-ring formation and coupling it to cell cycle progression. Models will be discussed for how PomZ identifies midcell.

ISV11
Integration of signals in the regulation of bacterial nitrogen assimilation

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In bacteria, nitrogen assimilation is coordinated with other aspects of metabolism and cellular energy status. Three major signals are known to control the expression of nitrogen regulated genes and the activity of the enzyme glutamine synthetase, which plays a key role in the assimilation of the preferred nitrogen source, ammonia. These three signals are (i) glutamine, (ii) a-ketoglutarate, and (iii) the ratio of ATP to ADP, which is an indicator of the cellular adenylylate energy charge. In this presentation, I will review our understanding of how these signals function to control nitrogen assimilation in *Escherichia coli*.
The expression of nitrogen-regulated (Ntr) genes in *E. coli* is controlled by a cascade-type system consisting of two linked covalent modification cycles, in which the downstream cycle is comprised of a two-component regulatory system that directly controls gene expression. The regulation of glutamine synthetase activity is also controlled by a cascade-type system comprised of two linked covalent modification cycles, in which the downstream cycle is comprised of glutamine synthetase and the enzyme that controls its activity by reversible adenylylation. The two signalling systems are connected, as the upstream cycle for both systems is the same. In this upstream covalent modification cycle, the PII signal transduction

protein is reversibly uridylylated by the signal transducing enzyme UTase/UR. The PII protein then regulates the activity of the two downstream covalent modification cycles. PII is one of the most widely distributed proteins in nature, and it appears to be universally involved in controlling nitrogen assimilation.
I will discuss biochemical studies that indicated that PII is the sensor of the a-ketoglutarate signal and of the adenylylate energy charge signal, which are antagonistic, and will review our current understanding of the signaling mechanisms. I will also discuss biochemical studies describing the sensation of glutamine by two of the signal-transduction enzymes of the system. Finally, I will review recent studies that revealed factors influencing the sensitivity of responses to the glutamine signal. Together, these results will provide a basic overview of the control of nitrogen assimilation in *E. coli*.

ISV12
Signalling in biofilm formation of *Bacillus subtilis*
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Cells of *Bacillus subtilis* can either be motile or sessile, depending on the expression of mutually exclusive sets of genes that are required for flagellum or biofilm formation, respectively. Both activities are coordinated by the master regulator, SinR. We have identified three novel factors that are required for biofilm formation, the transcription factor CcpA, the novel RNase Y and the previously uncharacterized YmdB protein. Since YmdB had not been studied before, we analyzed the corresponding mutant in more detail. We observed a strong overexpression of the *hag* gene encoding flagellin and of other genes of the SigD-dependent motility regulon in the *ymdB* mutant, whereas the two major operons for biofilm formation, *tapA-sipW-tasA* and *epsA-O*, were not expressed. As a result, the *ymdB* mutant is unable to form biofilms. An analysis of the individual cells of a population revealed that the *ymdB* mutant no longer exhibited bistable behavior; instead, all cells are short and motile. The inability of the *ymdB* mutant to form biofilms is suppressed by the deletion of the *sinR* gene encoding the master regulator of biofilm formation, indicating that SinR-dependent repression of biofilm genes cannot be relieved in a *ymdB* mutant. Our studies demonstrate that lack of expression of SlrR, an antagonist of SinR, and overexpression of SlrR suppresses the effects of a *ymdB* mutation.

ISV13
No abstract submitted!

ISV14
No abstract submitted!

ISV15
Suppression of *Clostridium difficile* disease and transmission by the intestinal microbiota
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The human large intestine plays host to an extremely abundant and diverse collection of microbes, which are collectively termed the intestinal microbiota. Under normal circumstances our resident microbes are considered to play a number of key roles in the maintenance of human health. One example is the establishment of a phenomenon termed “colonization resistance”. During health, or in the absence of antibiotic use, our indigenous microbiota can effectively inhibit colonization and overgrowth by invading “foreign” microbes such as pathogens. In doing so our microbiota helps to protect us from gastrointestinal infection and also acts to keep potentially pathogenic indigenous species such as *Clostridium difficile* under control. Colonization resistance against *C. difficile* is typically broken by broad-spectrum antibiotic use, which disrupts the density, composition and activity of the intestinal microbiota and allows the pathogen to proliferate in the intestine and cause disease. Using a mouse model of disease we monitored longitudinal shifts in microbiota composition in an attempt to better understand the underlying dynamics behind antibiotic-associated *C. difficile* infection and transmission. We find that infection with certain strains of *C. difficile* results in prolonged shedding of *C. difficile* spores, which occurs in tandem with inhibited re-establishment of colonization resistance. This leads to enhanced transmission of these strains and also mimics the situation observed in around 25% of *C. difficile* cases in humans where the disease becomes refractory to treatment and patients suffers constant relapses, even after treatment with strong antibiotics such as vancomycin. I will therefore describe more novel means of restoring bacterial diversity in the intestine and offer some perspectives on future challenges for developing therapies to promote colonization resistance.

ISV16
Systems biology of halophilic archaea
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Extreme halophiles from the branch of euryarchaeota live in very hostile environments characterized by intense radiation and shortage of nutrients and oxygen. *Halobacterium salinarum* became a model organism to study adaptation of life to these extreme conditions and cytoplasmic salt concentrations of up to 5 M. After a general description of halophilic features of these organisms specific example of systems biological models of intermediary metabolism, bioenergetics and signal transduction on the basis of -omics data as well ass biochemical and behavioural experiments will be presented.

ISV17
Microbial survival strategies: *Staphylococcus aureus* as a highly effective surviver
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S. aureus uses multiple strategies to survive from colonizing passively the host to attacking the host defenses. *S. aureus* has traditionally been considered a colonizer of the nose, but the newer methicillin resistant strains (MRSA) have the capacity to colonize the throat, vagina, rectum, and skin. Once the skin is barrier is breached host cationic antimicrobial proteins (CAPs) are released from the keratinocytes, but *S. aureus* has a two-component regulator, GraRS, which recognizes and confers resistance to CAPs. Local resident inflammatory cells such as macrophages and mast cells can be circumvented by the organism being taken up into the host cell via $\alpha5\beta1$ integrin and eventually the cytoplasm thereby avoiding the bactericidal mechanisms of these professional phagocytes. *S. aureus* has a very wide variety of factors that block each stage of influx of neutrophils (PMNs) into the area of local infection. Those PMNs that do reach the infected site can also have their bactericidal mechanisms circumvented so that they too become a reservoir for *S. aureus*. Proliferation of the staphylococci can result in local abscess formation wherein bacterial proteins such as coagulase can limit blood flow thereby reducing PMN influx, ClfA and Eap can help in the formation of an abscess, and the anaerobic micro-environment will also reduce the effectiveness of professional phagocytes. Other *S. aureus* can down-regulate their virulence factors by becoming small colony variants (SCVs) and deleting their *agr* and its associated virulence regulon. Although these organisms are much less aggressive, they are better able to enter a very wide variety of host cells (including respirator and mammar epithelial cells, endothelial cells, fibroblasts, and keratinocytes), and persist because they fail to lyse or to produce apoptosis of the host cells, do not stimulate hypoxia-inducible factor (HIF), and resist host cell CAPs. Phenotypic switching to SCVs has now been demonstrated in animal models, and these SCVs generate a much less robust immune response than their wild type parent strains. In addition, these apparently less virulent variants show increased expression of adhesins, thereby allowing them to persist better on host tissues. The ability of *S. aureus* to form biofilm is another mechanism for escaping host defenses. T cells have been recently implicated in the defense against *S. aureus* infections, and T-cell angery is found in chronic infections. Particularly worrying is the fact that multi-drug resistant strains are now circulating that have enhanced ability to survive on skin, in the lung, and kidneys. For example, resistance to linezolid has been linked to point mutations in *relA* that allowed for the development of SCVs that showed an enhanced stringent response and persistent infection in patients and animal models. The success of *S. aureus* as a pathogen certainly relates to the vast array of survival strategies.

BDV001
Setting the pace: Mechanisms controlling the temporal regulation of the *Caulobacter crescentus* cell cycle
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One of the most fundamental processes in biology is the regulation of the cell cycle, involving DNA replication, chromosome segregation, and cell division. The alpha-proteobacterium *Caulobacter crescentus* has emerged as an excellent model for studying the basic principles of cell cycle control, largely owing to an ability to synchronize large populations of cells. Additionally, *Caulobacter* divides asymmetrically, yielding daughter cells that differ with respect to their replicative fates and morphology. This intrinsic asymmetry has also made *Caulobacter* an attractive model for understanding spatial regulatory mechanisms. Our recent work

demonstrated that the *Caulobacter* cell cycle is composed of two separable control modules [1]. One module centers on an essential DNA-binding protein called CtrA, which governs replicative asymmetry, polar morphogenesis and cell division. The other module centers on the replication initiator DnaA and dictates the periodicity of DNA replication, thereby acting as an intrinsic pacemaker of replication and the cell cycle. Although CtrA regulation is now understood in great detail, our understanding of the mechanisms governing DnaA activity remains incomplete. Using a combination of genetic and yeast two-hybrid screens we have identified a novel regulator of DnaA, whose precise role in the regulation of DnaA and replication are currently under investigation. Dissecting the regulation of the cell cycle in *Caulobacter* and understanding how it relates to the cell cycles of other bacteria will ultimately provide insight into how the bacterial cell cycle has evolved to allow cells to grow and proliferate in diverse environmental niches.

[1] Jonas K, Chen YE, Laub MT. (2011). Modularity of the bacterial cell cycle enables independent spatial and temporal control of DNA replication. Current Biology. 21(13):1092-101.

BDV002

How to generate a protein gradient within a bacterial cell: dynamic localization cycle of the cell division regulator MipZ

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Intracellular protein gradients play a critical role in the spatial organization of both prokaryotic and eukaryotic cells, but in many cases the mechanisms underlying their formation are still unclear. Recently, a bipolar gradient of the P-loop ATPase MipZ was found to be required for proper division site placement in the differentiating bacterium Caulobacter crescentus.

MipZ interacts with a kinetochore-like nucleoprotein complex formed by the DNA partitioning protein ParB in proximity of the chromosomal origin of replication. Upon entry into S-phase, the two newly duplicated origin regions are partitioned and sequestered to opposite cell poles, giving rise to a bipolar distribution of MipZ with a defined concentration minimum at the cell center. Acting as a direct inhibitor of divisome formation, MipZ thus effectively confines cytokinesis to the midcell region.

Based on the crystal structures of the apo and ATP-bound protein and by means of mutant variants of MipZ, we dissected the role of nucleotide binding and hydrolysis in MipZ function. Gradient formation is found to rely on a nucleotide-regulated alternation of MipZ between a monomeric and dimeric form. MipZ monomers interact with ParB, which results in recruitment of MipZ to the polar regions. Our results suggest that the polar ParB complexes locally stimulate the formation of ATP-bound MipZ dimers, the biological active form that inhibits FtsZ assembly. Moreover, dimers are retained near the cell poles through association with chromosomal DNA. Due to their intrinsic ATPase activity, dimers eventually dissociate into freely diffusible monomers that undergo spontaneous nucleotide exchange and are recaptured by ParB.

The MipZ gradient can thus be envisioned as an asymmetric distribution of dimers that are released from a polar pool and slowly diffuse towards mid-cell. By virtue of the marked differences in the interaction networks and diffusion rates of monomers and dimers, ATP hydrolysis promotes oscillation of MipZ between the polar ParB complexes and pole-distal regions of the nucleoid. The MipZ gradient thus represents the steady-state distribution of molecules in a highly dynamic system, providing a general mechanism for the establishment of protein gradients within the confined space of the bacterial cytoplasm.

BDV003

Regulation of cellular reversals in *Myxococcus xanthus*

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Social behaviour patterns, such as predation or the formation of fruiting bodies in the soil bacterium *Myxococcus xanthus*, require the coordinated movement of cells. Myxococci lack flagella, but move by gliding on solid surfaces using two genetically distinct mechanisms: social S-motility mediates movement in groups while adventurous A-motility powers individual cells.

In both systems, coordinated movement is achieved by regulating the frequency of cellular reversals. Reversals involve the inversion of the cell´s polarity axis, which is established by a pair of GTPase/GAP proteins (MglA and MglB) that localize to opposite cell poles. MglA and MglB switch their position at reversal, resulting in the re-orientation of the S- and A-motility motors.

The frequency of cell reversals is modulated by the Frz signalling pathway, which operates similar to the *E. coli* chemotaxis system. In the presence of a chemoreceptor homologue FrzCD and a coupling protein FrzA, phosphotransfer occurs from the FrzE histidine kinase to a response regulator, FrzZ. We currently use genetic and biochemical approaches to identify downstream targets of the response regulator FrzZ. Characterizing the output of the pathway is essential to understand how the Frz system controls the GTPase/GAP switch and times cell reversals.

BDV004

The cell wall amidase AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme* ATCC 29133

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Filamentous cyanobacteria of the order Nostocales are primordial multicellular organisms, a property widely considered unique to eukaryotes. Their filaments are composed of hundreds of mutually dependent vegetative cells and, when deprived for a source of combined nitrogen, regularly spaced N_2-fixing heterocysts. Furthermore, specialized spore-like cells (akinetes) and motile filaments (hormogonia) differentiate under certain environmental conditions. The cells of the filament exchange metabolites and signaling molecules, but the structural basis for cellular communication within the filament remains elusive.

Here we show that mutation of a single gene, encoding cell-wall amidase AmiC2, completely changes the filamentous morphology of *N. punctiforme* and abrogates cell differentiation and intercellular communication. The mutant forms irregular clusters of twisted cells connected by aberrant septa. Rapid intercellular molecule exchange takes place between cells of the wild-type filaments, but is completely abolished in the mutant, and this blockage obstructs any cell-differentiation, indicating a fundamental importance of intercellular communication for cell-differentiation in *N. punctiforme*. AmiC2-GFP localizes in the cell wall implying that AmiC2 processes the newly synthesized septum into a functional cell-cell communication structure during cell division. Ultrastructural analysis shows a contiguous murein sacculus with individual cells connected by a single-layered septal cross-wall in the mutant as well as in the wild type. AmiC2-GFP also accumulates in the region of the polar neck during heterocyst differentiation and disappears after heterocyst maturation as well as in the septa of mature akinetes. Synchronously dividing cells of hormogonia accumulate AmiC2-GFP in the septal cross walls. The AmiC2 protein could be expressed in *E. coli* and purified. It shows cell wall lytic activity and can complement the filamentous phenotype of *E. coli* triple amidase mutants.

From our studies we can conclude that the cell wall amidase AmiC2 of *N. punctiforme* is a novel morphogene required for cell-cell communication, cellular development and multicellularity in this cyanobacterium

BDV005

Functional analysis of cytoskeletal proteins implicated in magnetosome formation and cell division in *Magnetospirillum gryphiswaldense*

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Magnetotactic bacteria use magnetosomes to move along magnetic field lines. Magnetosomes are organelles which consist of membrane-enclosed nanometer-sized magnetite crystals lined up along the cell axis. This magnetosome chain is located at midcell and split during cell division, whereupon magnetosomes are thought to re-localize from the new cell poles to the new centres by an as yet unknown mechanism. Midcell information in bacteria is usually provided by the essential cell division protein, FtsZ. Intriguingly, *M. gryphiswaldense* has two *ftsZ* homologs (a genuine *ftsZ* and *ftsZm*). *ftsZm* is co-located within the genomic magnetosome island with other magnetosome genes including *mamK*, which encodes a further, actin-like cytoskeletal protein that polymerizes into straight magnetosome filament structures. We analyzed the function of these cytoskeletal elements likely implicated in the magnetosome chain division and segregation process.

Fluorescence microscopy revealed that $FtsZ_{Mgr}$ and FtsZm co-localize at the division plane in asymmetric spots opposite to the MamK filament. This asymmetry coincides with an asymmetric indentation and division of *M. gryphiswaldense* cells which likely facilitates cleavage of the magnetosome chain owing to leverage. In contrast to previous observation, deletion of had no effect on magnetite crystal biomineralization but influenced the cell size of *M. gryphiswaldense*. To analyze the dynamics of magnetosome daughter chain segregation we performed fluorescence time lapse microscopy of growing cells. Our preliminary results suggest that equal proportions of magnetosomes are rapidly removed from the division plane and become trapped at the centres of future daughter cells during the division process. Electron microscopy of division-inhibited cells suggests

that this trapping depends on the actin-like MamK protein. Overall, our data suggest that magnetosome segregation and re-localization is tied to an active, divisome and MamK-dependent mechanism.

BDV006

The PomX protein is required for cell division in *Myxococcus xanthus*

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FtsZ is a highly conserved component of the bacterial cell division machinery and formation of the FtsZ-ring at the incipient division site is one of the earliest detectable event in the assembly of the division machinery. In bacteria selection of the site of cell division has been thought to rely on negative regulators only; however, we recently showed that the ParA-like protein PomZ positively regulates Z-ring formation in *Myxococcus xanthus*. Briefly, in a *pomZ* mutant FtsZ-ring formation is strongly reduced and the FtsZ-rings formed are abnormally positioned. PomZ localization changes with cell cycle progression culminating in localization to the incipient division site before and in the absence of FtsZ.*In vitro* FtsZ of *M. xanthus* hydrolyses GTP but do not assemble into filaments suggesting that GTP hydrolysis-dependent depolymerization is as fast as the GTP-dependent polymerization, thus, precluding filament accumulation. PomZ weakly stimulates FtsZ polymerization suggesting that PomZ functions to directly recruit FtsZ to midcell and to stabilize the Z-ring. Thus, PomZ provides direct positional information for Z-ring formation, thereby, positively regulating positioning of the division site.
To identify proteins important for directing PomZ to mid-cell, we focussed on the gene flanking *pomZ*, i.e. *pomX*, which encodes a protein with a C-terminal coiled-coil region. A *pomX* mutant phenocopies a *pomZ* mutant indicating that PomX is also involved in cell division. Consistently, in the absence of PomX, FtsZ-ring formation is significantly reduced and the Z-rings formed are abnormally localized. mCherry-PomX localizes in a cell cycle-dependent manner: In short cells, PomX forms a cluster away from mid-cell, and in longer cells a mid-cell cluster. Moreover, in the absence of PomX, PomZ localization to the off-center cluster and at mid-cell is abolished and in the absence of PomZ, PomX predominantly localizes randomly and rarely at mid-cell. Additionally, using purified His_6-tagged PomX protein PomZ was pulled out from wild type extracts. Moreover, His_6-PomX forms filaments in a cofactor-independent manner. According to our current working hypothesis PomX and PomZ interact to form a complex with FtsZ in that way fulfilling two purposes, recruitment of FtsZ to mid-cell and stabilization of the Z-ring.

BDV007

Cell differentiation in biofilms communities of *Staphylococcus aureus*

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Microbial communities embedded in biofilms generally differentiate into diverse subpopulations of specialized cells [2]. Development of biofilms relies on the spatio-temporal distribution of each one of the constituent subpopulations of specialized cells [3]. The pathogen Staphylococcus aureus is considered an important model to study biofilm development due to its ability to generate biofilm-mediated chronic infections [1]. Albeit the presence of specialized cells has been reported in communities of S. aureus [4] it is unknown whether biofilm formation in S. aureus requires the differentiation of specialized cell types and if so, what would be the contribution of those subpopulations to biofilm development.
We have developed a new model to study biofilm formation in which S. aureus forms extremely robust biofilms. This is based on the fact that biofilm development can be observed when cells grow on agar surfaces. In these conditions, the biofilms formed by S. aureus exhibits a sophisticated architecture that correlates with the strains' ability to form biofilm in vivo. Moreover, transcriptional reporters of genes known to be essential for biofilm development were created to visualize and monitor their expression pattern within the microbial community that conforms the biofilm. Examination of the expression of these reporters during biofilm formation showed a heterogeneous expression pattern among the community. A subpopulation of cells specialized in producing and secreting the polysaccharidic extracellular matrix differentiates. Differentiation of this subpopulation is dynamic since the proportion of the specialized cells varies along the different stages of the development. Similar pattern was observed for the subpopulation of cells responsible for the synthesis of adhesion proteins. Flow cytometry was used to quantify the temporal differentiation pattern of these subpopulations involved in biofilm formation.

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BDV008

Eating and being eaten: What bacterial cell biology can tell us about eukaryogenesis

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Prokaryotes are defined as a group of organisms generally lacking a membrane-bound nucleus or other membrane-bound organelle; these are the hallmarks of eukaryotic cells. Yet, species of the bacterial phylum *Planctomycetes* have been shown to harbor intra cytoplasmic membranes (ICM). The ICM of the planctomycetal model organism *Gemmata obscuriglobus* forms two double membranes surrounding the DNA in a nucleus-like compartment. In addition, some *Planytomycetes* divide like yeasts, via budding. They also lack the characteristic bacterial division protein FtsZ. Furthermore, planctomycetal membrane coat-like proteins resembling eukaryotic clathrins were recently discovered. Their involvement in vesicle formation and endocytosis-like uptake of proteins has been demonstrated. Consequently ancestors of modern *Planctomycetes* might have contributed to the origin of the eukaryotic cell plan. However, ultimate proof of endocytosis has been hampered by the lack of genetic tools for *Planctomycetes*. To overcome these limitations, we first screened for a suitable model organism among planctomycetal species available as axenic cultures. We identified *Planctomyces limnophilus* as a potential candidate and demonstrated that *P. limnophilus* displays the characteristic subcellular compartmentalization of the *Planctomycetes*. This finding provided us with the necessary impetus to develop genetic tools for its manipulation. Such tools make *P. limnophilus* relevant as a model for investigating the molecular basis of planctomycetal compartmentalization in general and to unearth the secrets of the planctomycetal impact on eukaryogenesis.

BDP001

The *Streptomyces* spore wall synthesizing complex SSSC

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The Mre-proteins of rod-shaped bacteria form a peptidoglycan (PG) synthesizing complex at the lateral wall to ensure elongation growth. Although mycelial *Streptomyces coelicolor* grows by apical tip extension which does not involve lateral cell wall synthesis, it contains three *mreB*-like genes and a complete *mreB* cluster comprising mreBCD, *pbp2* and *sfr* (*rodA*). Mutant analysis demonstrated that the *mre*-genes were not required for vegetative growth but affected sporulation. Mutant spores suffered from a defective spore wall rendering the spores sensitive to high osmolarity, moderate heat and to cell wall damage by lysozyme or vancomycin[1,2]. Study of protein-protein interactions by a bacterial two-hybrid analysis revealed a similar interaction pattern as reported for the lateral wall synthesizing complex suggesting that the *Streptomyces* spore wall is synthesized by a multi-protein complex which resembles the lateral wall synthesizing complex of rod-shaped bacteria[2]. Screening of a genomic library identified several additional interaction partners as novel components of the SSSC. Interaction of MreC, MreD, PBP2 and Sfr with the eukaryotic type Ser/Thr kinase SCO4078 indicates regulation of the SSSC by protein phosphorylation. Knock out experiments confirmed the role in spore wall synthesis for SCO2097, a small actinomycetes specific membrane protein, localized within the *dcw* cluster involved in cell division and PG synthesis, and *SCO2584* which is located next to teichoic acid biosynthetic genes. Since *ΔtagF*(*SCO2997*) and *ΔSCO2584* mutants showed a similar morphological defect as the *mre*-mutants, teichoic acids might also be involved in spore wall synthesis of *S. coelicolor*.

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BDP002

Magnetosome chains are recruited to cellular division sites and split by asymmetric septation

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Magnetotactic bacteria navigate along magnetic field lines using well-ordered chains of membrane enclosed magnetic crystals, referred to as magnetosomes,which have emerged as model to investigate intracellular

differentiation and organelle biogenesis in prokaryotic systems. To become divided and segregated faithfully during cytokinesis, the magnetosome chain has to be properly positioned, cleaved and separated against intrachain magnetostatic forces. Here we demonstrate that magnetotactic bacteria use dedicated mechanisms to control the position and division of the magnetosome chain, thus maintaining magnetic orientation throughout divisional cycle. Using electron and time-lapse microscopy of synchronized cells of *Magnetospirillum gryphiswaldense*, we demonstrate that magnetosome chains undergo a dynamic pole-to-midcell translocation during cytokinesis. Nascent chains were recruited to division sites also in division-inhibited cells, but not in a *mamK* mutant, indicating an active mechanism depending upon the actin-like cytoskeletal magnetosome filament. Cryo-electron tomography revealed that both the magnetosome chain and the magnetosome filament are split into halves by asymmetric septation and unidirectional indentation, which we interpret in terms of a specific adaptation required to overcome the magnetostatic interactions between separating daughter chains. Our study demonstrates that magnetosome division and segregation is coordinated with cytokinesis and resembles partitioning mechanisms of other organelles and macromolecular complexes in bacteria.

BDP003

Differentiation of bacterial spores by Fourier transform infrared spectroscopy (FTIR) and chemometrical data treatment

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Fourier transform infrared spectroscopy (FTIR) has been used as analytical tool in chemistry for many years to elucidate chemical structures. In addition, FTIR can also be applied as a rapid and non-invasive method to detect and identify microorganisms. The specific and fingerprint-like spectra allow - under optimal conditions - discrimination down to the species level. The aim of this study was to develop a fast and reproducible non-molecular method to differentiate *Bacillus* spores originating from different species as well as to identify spores in a simple matrix, such as the clay mineral, bentonite. We investigated spores from pure cultures of seven different *Bacillus* species by FTIR in reflection or transmission mode followed by chemometrical data treatment. All species investigated (*B. atrophaeus, B. brevis, B. circulans, B. lentus, B. megaterium, B. subtilis, B. thuringiensis*) are typical aerobic soil-borne spore formers. To simulate soil, mixtures of bentonite and spores of *B. megaterium* at various wt/wt ratios were included in the study. Both hierarchical cluster analysis and principal component analysis of the spectra along with multidimensional scaling allowed the discrimination of different species and spore-matrix-mixtures. Our results show that FTIR spectroscopy is a fast method for species-level discrimination of *Bacillus* spores. Spores were still detectable in the presence of the clay mineral bentonite. Even a tenfold excess of bentonite (corresponding to 2.1 x 10exp10 colony forming units per gram of mineral matrix) still resulted in an unambiguous identification of *B. megaterium* spores.

BDP004

Functional complementation of large operon deletions within the magnetosome Island of *Magnetospirillum gryphiswaldense*

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The magnetotactic bacterium *Magnetospirillum gryphiswaldense* produces intracellular organelles, the magnetosomes, which consist of magnetite crystals surrounded by a magnetosome membrane. Their uniform sizes and unique magnetic properties make them highly attractive for biotechnological and medical applications. Most of the genes controlling magnetosome formation have been identified within a genomic magnetosome island (MAI) of 115 kb. By mutational analysis, the *mamAB*, *mamGFDC*, *mms6* and *mamXY* operons, which have sizes between 2 and 17 kb and which comprise 30 genes in total, were implicated in the synthesis of properly sized and shaped magnetosomes. However, complementation of operon mutants has proven difficult due to the requirement to clone, transfer and express large genomic fragments. Complementation of smaller regions up to 5 kb (*mamGFDC*, *mamXY*) was accomplished by conjugational transfer of replicative plasmids, resulting in stable in trans expression and reconstitution of wildtype phenotypes. For cloning of larger fragments, comprising for example the large *mamAB* operon encoding 17 magnetosome genes, we used recombinogenic cloning. Conjugational transfer of replicative vectors harboring this region, however, revealed high instability of the plasmids and resulted in partial degradation of cloned genomic fragments, probably due to toxic effects of multicopy expression of encoded magnetosome membrane proteins. Therefore, alternative strategies, such as expression in RecA$^-$ background strains, use of inducible expression systems, and chromosomal insertion are currently investigated for stable expression. Eventually, cloning and functional expression of entire large operons from *M. gryphiswaldense* might be also useful for future metabolic engineering of the magnetosome synthesis pathway.

BDP005

A small acid soluble spore protein is essential for germination of *Clostridium acetobutylicum* spores

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Clostridium acetobutylicum is a potent solvent producer and in recent years it has become a model organism for the understanding of the molecular biology of non-pathogenic clostridia. We are interested in the events during the cell cycle of *C. acetobutylicum*. Here, we focus on results dealing with aspects of the resistance and germination capability of its spores.
Alpha/beta-type small acid soluble spore proteins (SASP) are usually located in the core of the endospores. They carry important functions like the protection of spore DNA against damage due to desiccation, heat or chemical agents. Furthermore, during germination fast degradation of SASPs by germination specific proteases provide an important amino acid pool for the development of the new vegetative cell.
In the genome of *C. acetobutylicum* five open reading frames are expected to encode SASP-like proteins [1]. To unravel the individual functions of these proteins we generated specific knock out mutants using the ClosTron Technology by insertional inactivation based on the selective retargeting group II intron [2]. Analysis of the phenotypes using transmission electron microscopy revealed the production of morphological intact spores. However, sporulation assays [3] proved that the individual germination capabilities of the mutant strains were affected to different levels. Most interestingly, one SASP was essential for germination which could be restored by a plasmid-based complementation of the gene knock out.
[1] Nölling *et al*., 2001, J Bacteriol. 183:4823-4838
[2] Heap *et al*., 2007, J Microbiol Methods 70:452-464
[3] Burns *et al*., 2010, J Bacteriol. 192:657-664

BDP006

Spore formation in *Clostridium acetobutylicum* ATCC 824 depends on granulose synthesis

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The transition phase of growth of the Gram-positive, spore-forming anaerobe Clostridium acetobutylicum is characterized by several morphological changes. At the beginning swollen and cigar shaped cells, clostridial stages, are formed. In the cells, a polymeric carbohydrate, granulose is accumulated in the form of granules. Granulose is expected to be a energy- and carbon storage, necessary as a prerequisite for sporulation.
We proved that a single glycogen synthase (GlgA) in the genome of C. acetobutylicum plays a crucial role in the biosynthesis of granulose. A glgA insertion mutant (ClosTron® technology, [1]) was unable to accumulate granulose and did not form endospores. Results of the phenotypic characterisation are presented. This data includes colony morphology, cell differentiation and sporulation assays ofglgA-mutant cells in comparison to the wildtype.
Detailed comparative TEM studies revealed that even prespore formation in the mutant strain seemed to be blocked at a very early stage. Molecular analysis confirmed the correct insertion into the target gene and a negative influence on granulose-gene specific mRNA formation. However, transcription of the master regulator of sporulation spo0A seemed not to be affected (RT-PCR). Almost every gene of the granulose metabolism was influenced, whereas first evidence could be gained, that missing granulose affects degradation by a feed-back mechanism.
[1] Heapet al.,2007, J Microbiol Methods 70:452-464

BDP007

Monitoring of population dynamics of *Corynebacterium glutamicum* by multiparameter flow cytometry

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Corynebacterium glutamicum is a Gram-positive soil bacterium that is used as an industrial amino acid producer. For strain analysis and process monitoring usually average data resulting from the analysis of bulks of cells are provided for key parameters such as growth rate, productivity, and viability. However, several studies of the last decades revealed that even isogenic bacterial populations may exhibit significant cell-to-cell variation due to differences in microenvironment, cell age, cell cycle or stochastic effects on gene expression. In this context, fluorescence-activated cell sorting (FACS) allows a rapid and efficient insight into complex phenotypes and allows high-throughput analysis at the single cell level.

Here, multiple parameters were analyzed in single cells of *Corynebacterium glutamicum* via FACS. By analyzing a typical growth curve of *C. glutamicum* subpopulations were identified differing in size, DNA pattern, metabolic activity, membrane integrity, and membrane potential. These populations show a dynamic pattern depending on strain background, cultivation conditions, and growth phase. DNA patterns oscillate within the growth curve. Cells in the early log phase contain mainly a single chromosome equivalent followed by a proliferation phase characterized by a decrease in cells with a single chromosome equivalent and an increase in cells with multiple chromosome equivalents. Cells in the stationary phase exhibit predominantly a single chromosome equivalent. The reductase activity (indicator for electron transport chain function and cellular viability) also shows significant correlation with cell densities. Cells of the log phase show the highest reductase activity whereas cells of the early or late log phase exhibit a reduction in viability; stationary cells demonstrate the lowest activity as well as a depolarization of the cell membrane which could not be detected in cells of the log phase. The rate of depolarization correlates with the uptake of propidium iodide indicative for damaged cell membranes. These results demonstrate flow cytometry as an efficient tool for the study of bacterial population dynamics and process monitoring at single cell resolution.

BDP008

Polar magneto-aerotaxis in *Magnetospirillum gryphiswaldense*

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The paradigmatic concept of random walk motion observed in most prokaryotes is greatly simplified in freely swimming magnetotactic bacteria (MTB) which contain a chain of nano-sized magnetic particles. Passive alignment with the Earth's magnetic field forces the bacteria onto a nearly linear track. In addition, most MTB possess the selectable trait to follow the magnetic field lines in a preferred swimming direction (either N- or S-seeking) depending on the prevailing habitat conditions. To date, the underlying molecular mechanism of how magnetic polarity is integrated with other taxis mechanisms is not understood.
M. gryphiswaldense is a bipolarly flagellated gradient organism which is capable of polar swimming behaviour if grown under selective conditions. Automated video tracking of wild type cells revealed swimming episodes in alternating directions which are interrupted by short reversals. The reversal frequency did not change significantly in polarised cultures.
We identified four chemotaxis gene clusters containing conserved genes cheAWYBR in the genome of *M. gryphiswaldense*. Whereas deletion of operons 2-4 did not impact on chemotaxis, only loss of CheOp1 had a clear effect on aerotaxis. This indicated a possible link between polarity and chemotaxis at the genetic level. In magnetospirillum cells having an apparently symmetrical morphology, polarity might be established by asymmetric localization of constituents of the chemotaxis machinery. Therefore, we studied the intracellular localization of fluorescent protein fusions to chemotaxis proteins CheA and CheW. Since these proteins localised to variable positions in the cell they are unlikely to determine polarity.
Swimming polarity is currently being studied quantitatively by a microfluidic assay using fluorescence-labelled cells and in competition assays. This will also reveal the putative selective advantage of magnetotaxis.

BDP009

Spatiotemporal patterns of microbial communities in a hydrologically dynamic alpine porous aquifer (Mittenwald, Germany)

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It has been repeatedly shown for aquatic habitats that microbial communities underlie seasonal dynamics and follow environmental gradients. Recently, microbial communities of karst aquifers were shown to be influenced by seasonal hydrodynamics. In turn, we investigated seasonal patterns of selected microbial and physical-chemical variables in groundwater and sediments of an alpine oligotrophic porous aquifer over a period of two years. Characterized by a high hydraulic conductivity and groundwater flow velocities, this aquifer exhibited pronounced seasonal hydrological dynamics, which are confirmed by pronounced groundwater table fluctuations. The groundwater table was found highest during summer along with lowest bacterial diversity (H' = 1.31 ± 0.35 SD) in suspended bacterial communities, as analyzed by T-RFLP fingerprinting. A similar pattern was observed for the total number of planktonic bacteria, with lowest numbers in spring and summer (1.4×10^4 cells mL^{-1}) and highest values (2.7×10^5 cells mL^{-1}) during winter season. The ratio of total versus active cells, determined by analysis of intracellular ATP, was lowest in summer and winter (0.07%~10%) and highest in autumn (16%~85%). Bacterial carbon production measurements revealed highest activities in summer and lowest in winter, with average carbon production of 6.22 and 1.30 ng C $L^{-1}h^{-1}$ respectively. The carbon turnover related to concentrations of AOC, which ranged from 5 to 25 μg L^{-1}, accounting for only 0.1 to 1.3% of the bulk DOC. Sediment bacterial communities from a nearby river exhibited a stable community composition and diversity when exposed to groundwater for one year. Initially sterile sediments, on the other hand, were readily colonized and established a bacterial diversity similar to the exposed river sediment. In conclusion, hydrodynamics markedly influenced the planktonic bacterial communities while attached communities have not been affected by the serious hydrological changes.

BDP010

During stress Spx hits the emergency brake on swimming motility

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Clp proteases are key players in the regulation of bacterial differentiation processes, such as competence [4] and sporulation [3]. The protease ClpXP is required for differentiation into motile cells in *Bacillus subtilis* [1]. An important proteolysis substrate of ClpXP is the global transcriptional regulator Spx, which activates the expression of stress tolerance genes during oxidative stress. At the same time Spx acts as a negative regulator of a distinct group of genes, including those responsible for competence development. Under non-stress conditions, Spx is efficiently degraded by the ClpXP protease, resulting in a low steady state level of the protein. However, in response to oxidative stress, this proteolysis is halted and Spx accumulates in the cell [2]. We have investigated the effect of ClpXP on swimming motility and found that the ClpXP substrate Spx acts as a negative regulator of flagellar genes. Furthermore, motility genes are transiently down-regulated in response to oxidative stress. We propose that during adverse environmental conditions, such as oxidative stress, the execution of the stress response program is given priority over motility development by the action of the regulator Spx. Possible mechanisms of this negative regulation are discussed.

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BDP011

Subcellular compartmentalization of a bacterial organelle by protein diffusion barriers

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Intracellular compartmentalization by different diffusion barrier mechanisms has previously been thought to be solely utilized by eukaryotic cells. Here, we report for the first time that non-membranous protein diffusion barriers also exist in prokaryotes. Using *Caulobacter crescentus* as a model organism, we show that these diffusion barriers physically separate cell envelope components of the cell body from the thin stalk appendage and create intra-stalk domains. The *Caulobacter* stalk represents a thin extension of the cell envelope that is free of DNA, ribosomes and most cytoplasmic proteins. It is segmented at irregular intervals by so-called crossbands, disk-like structures of so far unclear function and identity.
In this study, we discovered that crossbands serve as protein diffusion barriers. The major constituents of these diffusion barriers are four proteins that co-assemble in a cell cycle-dependent manner into a static complex at the junction between the stalk and the cell body. Using fluorescence loss in photobleaching (FLIP) we observed that, in contrast to eukaryotic cells, these diffusion barriers not only laterally compartmentalize cellular membranes but also limit the free diffusion of soluble (periplasmic) proteins. Moreover, competition assays with wild-type and barrier-deficient cells revealed that diffusion barriers are essential for fitness as they minimize the effective volume of the cell body envelope, thereby allowing faster adaptation to environmental changes that require the upregulation of protein production.
Collectively, our findings demonstrate that crossband formation in the stalked alpha-proteobacterium *Caulobacter crescentus* presents a novel mechanism to optimize growth by restricting protein mobility in a prokaryotic cell.

BDP012

Functional analysis of the magnetosome island in *Magnetospirillum gryphiswaldense*: The *mamAB* operon is sufficient for magnetite biomineralization

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The magnetotactic bacterium *M. gryphiswaldense* synthesizes intracellular membrane-enveloped crystals of magnetite, which serve for magnetotactic navigation. The biosynthesis of the nanometer-sized magnetosomes is under strict genetic control resulting in well-defined sizes and morphologies. Almost all genes implicated in the biogenesis were found located in a conserved genomic magnetosome island (MAI). Beside these *mam* and *mms* operons this 115-kb region is coding numerous transposases as well as hypothetical proteins of unknown functions. Therefore, we implemented a combination of comprehensive and functional analysis of all MAI proteins. By the construction of multiple large deletion mutants up to 59 kb we demonstrated that the MAI can be deleted without any consequences for growth under laboratory conditions. While the majority of MAI genes have no detectable function in magnetosome formation and could be eliminated without any effect, only <25% of the region comprising four major operons could be associated with magnetite biomineralization and coinciding with protein expression. Whereas deletion of *mms6*, *mamGFDC*, and *mamXY* operons lead to severe defects in morphology, size and chain assembly of magnetite crystals, only deletion of the large *mamAB* operon (16.4 kb) resulted in the complete loss of magnetic particles. However, even strains with multiple deletions including various combinations of the small *mms6*, *mamGFDC*, and *mamXY* operons (2-5 kb) retained the ability to synthesize small irregular crystallites. This demonstrates that whereas the *mamGFDC*, *mms6*, and *mamXY* operons have crucial and partially overlapping functions for the formation of functional magnetosomes, the *mamAB* operon is the only essential operon for magnetite biomineralization and also sufficient even in the absence of the highly conserved magnetosome operons. Our data further reduce the known minimal gene set required for magnetosome formation and will be useful for future genome engineering approaches.

BDP013

Magnetosome expression of heterologous fusion proteins in *Magnetospirillum gryphiswaldense*

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Magnetosomes are membrane-enclosed magnetic organelles produced by magnetotactic bacteria. The biomineralization of these unique nanostructures, which consist of magnetite (Fe_3O_4) crystals, is under strict genetic control. Thus, genetic engineering can be employed for the biosynthesis of magnetic nanoparticles with tuned characteristics.
Due to encapsulation of the magnetite core within the magnetosome membrane, also the magnetosome surface is accessible to functionalization by genetic engineering, which makes magnetosomes an interesting and promising tool in fields like (bio)nanotechnology, synthetic biology or even theranostic nanomedicine.
The mamC protein is favorable magnetosome anchor because of its abundance within the magnetosome membrane. Using mamC as entrenchment, we analyzed the magnetosome expression of various foreign proteins.
To secure high yields of functional magnetosome fusions and reduce potential toxic effects of foreign proteins, an inducible system for high expression is constructed for the production of functionalized magnetosomes. To this end, several hybrid promoters are currently analyzed in the model organism *Magnetospirillum gryphiswaldense*. Using GFP as a reporter for heterologous expression, expression was optimized by adjusting codon usage and GC content, which resulted in a significant increase of the fluorescence and protein abundance. Achieving a high and stable expression of GFP fusions is desirable because they are an important tool for tracking of intracellular protein localization during magnetosome synthesis.
Currently, besides GFP, fusions with other proteins are being explored, including biomolecular coupling groups as well as nanobodies and biomineralizing peptides.

BDP014

Metagenomic cloning and single cell sorting reveals unknown diversity of uncultivated magnetotactic bacteria from marine and freshwater habitats

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Magnetotactic bacteria (MTB) are a diverse group of prokaryotes which orient along magnetic fields using membrane-coated magnetic nanocrystals of magnetite (Fe_3O_4) or greigite (Fe_3S_4), the magnetosomes. Previous phylogenetic analysis of MTB has been limited to few cultivated species and most abundant members of natural populations, which were assigned to *Proteobacteria* and the *Nitrospirae* phyla. Recent analysis of low-abundant MTB using single-cell techniques and whole genome amplification (WGA) revealed the existence of MTB, also in the deep branching candidate division OP3. Here, we used analysis of 16S rDNA clone libraries of uncultivated magnetically collected MTB. This was combined with targeted phylogenetic and ultrastructural analysis of individual microsorted magnetically responsive cells followed by WGA and ultrastructural analysis. Application of this approach to freshwater and marine sediment samples revealed extensive and novel diversity of MTB. By single-cell analysis three different morphotypes present in a marine sediment could be assigned to three phylotypes, and seven morphotypes in a freshwater samples to six phylotypes, whereas clone libraries revealed 48 and 96 anonymous clones in the marine and freshwater sample, respectively. Partial overlap between both techniques indicated that a combination of both methods is most efficient for maximum recovery of MTB diversity. Most of the newly identified MTB belonged to various lineages of *Proteobacteria* and the *Nitrospirae* phylum. By PCR screening of small insert libraries from WGA amplified DNA also genes implicated in magnetosome synthesis could be identified from some of the microsorted cells, indicating that single-cell based approach is also potentially useful for diversity analysis of functional genes. In conclusion, the combination of metagenomic cloning and single cell sorting represents a powerful approach to recover maximum bacterial diversity including low-abundant magnetotactic phylotypes from environmental samples and may also provide access to metagenomic analysis of uncultivated MTB.

BDP015

Characterization of PomY, a regulator of FtsZ localization in *Myxococcus xanthus*

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Chromosome segregation and cell division are tightly regulated in bacteria to ensure the precise distribution of chromosomes to daughter cells and that daughter cells have appropriate sizes. In bacteria, cell division depends on assembly of the tubulin homologue FtsZ into a circumferential ring-like structure, the Z-ring, at the incipient division site and regulation of division site positioning occurs at the level of Z-ring positioning. We previously showed that PomZ and FtsZ co-localize at mid-cell in *Myxococcus xanthus*and that PomZ arrives earlier than and independently of FtsZ at mid-cell. Moreover, we observed that PomZ stimulates FtsZ polymerizationin vitro. On the basis of these observations, we have hypothesized that regulation of FtsZ localization to the incipient division site is positively regulated in *M. xanthus* by PomZ. However, how PomZ finds mid-cell is not clear.
To identify proteins that regulate PomZ localization and function, we analysed the gene upstream of *pomZ*, *pomY*,Mxan_0634. PomY consists of a domain with six HEAT-like repeats and a 110 residues coiled-coil region. A Δ*pomY* mutant has a phenotype similar to that of a *pomZ* mutant and generates filamentous cell with multiple chromosomes and chromosome-free minicells. Importantly, Δ*pomY* mutant is indistinguishable from WT cells with respect to chromosome replication and segregation suggesting a direct role for PomY in cell division. Accordingly, we observed that PomY is important for formation of the Z-ring and for the correct mid-cell positioning of the Z-ring.*In vivo* studies showed that the localization of an active PomY-mCherry fusion correlates with cell length and cell cycle: PomY-mCherry localization is dynamic and early during the cell cycle/in small cells it localizes over the unreplicated chromosome. Later during the cell cycle/in longer cells, PomY localizes at or close to mid-cell between segregating chromosome. In cells that have finished chromosome replication and segregation PomY-mCherry is at mid-cell, even before constrictions are observed. Moreover, PomY is required for correct localization of PomZ. Our data suggest that PomY is essential for normal cell division and binds at mid-cell early during the cell cycle and thereby help to mark the future division site.

BDP016

The paryphoplasm of Planctomycetes is a highly derived periplasm

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Planctomycetes are bacteria with an unusually high degree of intracellular compartmentalization. Although the extent of compartmentalization varies, the cell content of all planctomycetes is differentiated into at least a central riboplasm containing the genomic DNA and ribosomes, and an extensive peripheral compartment termed the paryphoplasm. Uniquely in bacteria, endocytotic protein uptake and membrane trafficking has been observed in the paryphoplasm of *Gemmata obscuriglobus*. As the division of the cellular contents into paryphoplasm and riboplasm is reminiscent of the division of the cell contents of Gram-negative bacteria into a central cytoplasm and a peripheral periplasm, the genome sequence of the model planctomycete *Planctomyces limnophilus* was examined for the presence of proteins involved in the maintenance and functioning of the Gram-negative periplasm and outer membrane. The *P. limnophilus* genome was found to encode a large number of proteins typical for the periplasm and the outer membrane, including the outer membrane insertion protein BamA and outer membrane components of pili and flagella. Few homologs of Gram-negative protein secretion systems were found, and very few proteins were found in the culture supernatant. In contrast, ~22% of all encoded proteins were predicted to carry a Sec signal peptide, which corresponds well with 20-30% of all proteins targeted to the periplasm in a typical Gram-negative bacterium. A comparison of these proteins with the periplasmic proteins of Gram-negative bacteria also revealed substantial functional overlap between the two sets. We propose that the paryphoplasm is derived from a modified and greatly expanded periplasm and discuss the role of this cellular compartment in the lifestyle of this group of organisms.

BDP017

Lipid specificity of a bacterial dynamin-like protein

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Membrane fusion and fission are rapid, dynamic processes that occur in eukaryotic and prokaryotic cells to facilitate generation and transport of vesicles, induce membrane trafficking, maintain cell shape and size. Proteins of dynamin superfamily play an important role in maintenance of membrane dynamics. This protein family includes members like classical dynamins, dynamin-related proteins and guanylate-binding proteins or atlastins. Dynamin GTPases demonstrate functions such as vesicle scission, division of organelles, cytokinesis and microbial resistance. DynA is a 136 KDa GTPase in *Bacillus subtilis*. Its structure is remarkable, as it seems to have developed from a fusion event between two molecules thus consisting of two separate GTPase and dynamin-like subunits. On account of sequence homology to other bacterial and eukaryotic dynamins, similar biochemical properties such as GTP hydrolysis and membrane fusion, DynA is classified as a member of the dynamin superfamily. It is a bacterial dynamin-like protein (BDLP) whose function is reasonably parallel to eukaryotic mitofusins, involved in mitochondrial outer membrane fusion. Mitofusins mediate nucleotide-dependent fusion whereas DynA shows nucleotide-independent membrane tethering and fusion in vitro. Our recent in vitro data has shown DynA to mediate nucleotide-independent fusion of vesicles generated from phosphatidylglycerol (PG) and cardiolipin (CA). Vesicle tethering but not fusion was observed with other lipids tested so far which is suggestive of DynA's affinity for PG and CA phospholipids. Currently we determine the amino acid positions in DynA that mediate such lipid specificity. This might allow identifying DynA's target on bacterial membrane. Overall aim of this project is revealing the function and actual mechanism of DynA in bacteria. *B. subtilis* DynA seems like a promising BDLP candidate due to the well characterised molecular biology of its host organism and the unique structural features of the molecule. Biochemical and cell biological characterisation of DynA using the simple *B. subtilis* may provide mechanistic implications in particular for the mitochondrial membrane dynamics as well as other dynamin-like proteins (DLPs).

BDP018

The *mamXY* operon is involved in controlling magnetite formation and magnetosome chain positioning in *Magnetospirillum gryphiswaldense*

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Magnetotactic bacteria (MTB) use intracellular chains of membrane-enveloped magnetite crystals, called magnetosomes, to orientate along magnetic fields. The sequential steps of magnetosome synthesis involve intracellular differentiation and include vesicle formation, magnetite nucleation and mineralization as well as magnetosome chain alignment and are subject to tight genetic regulation. Most of the genes implicated in magnetosome formation are organized in four operons that are clustered within a genomic magnetosome island. Despite of recent progress in characterization of these genes, the function of the *mamXY* operon has not been well investigated so far. To close this gap, we created unmarked deletions of all four individual genes within this operon and analyzed the phenotype of the mutants. The *mamH-like* gene encodes for a unique membrane-spanning protein affiliated to the group of MFS transporters but fused to a putative ferric reductase-like domain. The Δ*mamH-like* mutant forms magnetite crystals with heterogenic size, structure and cellular distribution. The mutant also displays a delay in production of ferrimagnetic magnetosomes. A similar phenotype was observed upon deletion of *mamX*, indicating a function in the same cellular biomineralization process. Deletion of the MTB-specific *mamY* gene however, did not influence mineralization but led to mislocalization of magnetosome chains. Fluorescence microscopy revealed that MamY localizes as a filamentous structure coinciding with the expected position of the magnetosome chain. The protein may therefore directly participate in targeting magnetosomes to their assigned position by an as yet unknown mechanism. Unexpectedly, deletion of *ftsZm*, coding for a truncated homolog of the major cell division protein FtsZ, did not show any obvious cell division phenotype, and in contrast to previous reports also no biomineralization defects. In conclusion, our data suggests that the proteins encoded within the *mamXY* operon play a major role in magnetosome biomineralization and chain positioning.

BDP019

Mapping the interaction surfaces of the bacterial cell division regulator MipZ

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Proper positioning of the cell division site in *Caulobacter crescentus* is regulated by the ATPase MipZ, which forms bipolar gradients within the cell, thus restricting assembly of the cytokinetic FtsZ ring to the midcell region. Gradient formation is driven by a dynamic localization cycle that involves the alternation of MipZ between a monomeric and dimeric state with distinct interaction patterns and diffusion rates. This cycle depends on the oscillation of MipZ between non-specific chromosomal DNA and a polarly localized complex of the chromosome partitioning protein ParB. To map the surface regions that mediate the interaction of MipZ with FtsZ, ParB and DNA, we systematically exchanged surface-exposed residues using alanine-scanning mutagenesis. Analyzing the subcellular distribution of the mutant proteins as well as their ability to support division site placement, we identified three clusters of residues each of which is likely responsible for contacting one of the interacting proteins. Notably, the DNA-binding pocket of the MipZ dimer is composed of residues from both dimer subunits. Moreover, it was found to be located opposite the putative FtsZ-binding region, consistent with the previous finding that the regulatory effect of MipZ is specific for its dimeric form and involves contacts with both DNA and FtsZ. These results provide the first detailed analysis of the interaction determinants of MipZ and yield new insights into the mechanisms that underly the function of this unique regulatory system.

BDP020

Bactofilins: polar landmarks in *Myxococcus xanthus*

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Bacteria, similar to eukaryotes, possess cytoskeletons that are involved in the temporal and spatial organization of various cellular processes including cell division, cell morphogenesis, cell polarity, as well as DNA

partitioning. Out of these elements, the tubulin homologue FtsZ, the actin homologue MreB, and intermediate filament-like (IF) proteins are widespread in many bacterial lineages. In addition, in recent years, an increasing number of non-canonical cytoskeletons have been identified in bacteria. These include a new class of cytoskeletal proteins, named bactofilins, which was originally discovered in *Caulobacter crescentus*. Bactofilins are widely distributed among bacteria and show no similarity in either sequence or structure to other known cytoskeletal proteins. Interestingly, many species possess two or more bactofilin alleles, indicating multiple gene duplication events and functional differentiation. Previous work showed that in *C. crescentus*,two bactofilin paralogues, BacA and BacB, are involved in stalk biogenesis. In this study, we have extended the investigation of bactofilins to *Myxococcus xanthus*, a social species that contains four bactofilin homologues. Our results suggest that bactofilins of *M. xanthus* are involved in a variety of different processes, mediating the proper arrangement of protein complexes within the cell. Thus, bactofilins are a novel and widespread group of cytoskeletal proteins that show a conserved overall architecture but have diverged significantly with respect to their localization patterns and functions.

BDP021

Overexpression of Flotillins affects septum formation in *Bacillus subtilis*

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The model organism *Bacillus subtilis* has been traditionally used to study the presence of Flotillin proteins in bacteria (1-3). Flotillins are proteins that exclusively localize in lipid rafts of eukaryotic cells (4,5). In *B. subtilis*, flotillins localize in membrane microdomains that are functionally similar to the lipid rafts of eurkaryotes. This opens the door for using bacteria as systems to address intrincate questions in developmental biology such as the role of flotillins in lipid rafts or the influence that flotillins exerts on of diverse cellular processes that are related to lipid rafts.

We constructed a strain that simultaneously overexpress the two genes that encode for flotillin-like proteins in *B. subtilis*, yqfA and floT. Higher concentration of flotillin proteins was found in the membrane of growing cells. Remarkably, the overexpression of flotillin caused hyperactivation of several signaling transduction pathways associated with lipid rafts, like biofilm formation. Moreover, overexpression of flotillins caused aberrant cell division in *B. subtilis*. Cells showed smaller cell size, probably caused by the assembly of multiple septa along the cells, which eventually give rise to the formation of anucleate, non-autonomous minicells that swim freely in the cultures of *B. subtilis* (6). Microscopical and biochemical studies will be shown to elucidate how flotillin influence the proper localization of the proteins responsible for septum formation and the activation of the signaling pathway to biofilm formation in *B. subtilis*.

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BDP022

Differential expression of two flotillin-like proteins in *Bacillus subtilis*

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B. subtilisis a model organism traditionally used for the study of flotillin proteins in the membrane of bacteria (1-3). Flotillins are proteins exclusively associated with lipid rafts in eukaryotic cells (4-6). In *B. subtilis* and several other bacterial models, flotillin proteins localize in membrane microdomains that are functionally similar to lipid rafts of eukaryotic cells. Concretely, functional microdomains of *B. subtilis* contain two different flotillin-like proteins named YqfA and FloT. Since the role of flotillins in lipid rafts is not entirely clear, we used *B. subtilis* as model organism to carry out genetic and biochemical approaches in order to understand the role of each one of the two different flotillin-like proteins that are present in the functional microdomains.

Our data suggest that the absence of one of the flotillins does not affect the localization of the other in functional membrane microdomains of *B. subtilis*. The expression of FloT and YqfA flotilins is controlled differently because specific growing conditions lead cells to express just YqfA or both FloT and YqfA flotillins simultaneously. Expression of YqfA or FloT+YqfA in the functional microdomains of *B. subtilis* affects significantly the functionality of the signaling pathways harbored within the functional microdomains. Consistently with the different expression of the two flotillin-like proteins, our studies of gene expression using transcriptional reporters indicate that *floT* and *yqfA* genes are differently regulated. The regulation cascades that control the expression of both flotillin-encoding genes will be presented and discussed.

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CEV001

The bacterial MreB cytoskeleton organizes the cell membrane

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Many bacteria require the actin homolog MreB to maintain a rod-like cell shape[1, 2]. This bacterial cytoskeleton protein forms short filaments beneath the cell membrane and organizes lateral cell wall synthesis[1, 2, 3]. We found that compounds that perturb the localization of MreB also alter the lipid distribution in the cell membrane. Importantly, this effect leads to an aberrant distribution of membrane proteins. We show for the *E. coli* LacY permease and F_1F_o ATP synthase that this is accompanied by a reduction in enzyme activity. It appears that the MreB cytoskeleton, together with the transmembrane proteins MreC and MreD, actively organize the bacterial cytoplasmic membrane by forming fluid membrane micro-domains. This property is comparable to that described for the eukaryotic cortical actin cytoskeleton[4, 5]. We speculate that this common function of MreB and actin might be the reason why this protein family has remained conserved during evolution.

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CEV002

Cell envelope stress response in cell wall-deficient L-forms of *Bacillus subtilis*

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L-forms are cell wall-deficient cells that can grow and proliferate in osmotically stabilizing media [1]. Recently, a strain of the Gram-positive model bacterium *Bacillus subtilis* was constructed that allows a controlled switching between rod-shaped wild type cells and corresponding L-forms [2]. Both states can be stably maintained under suitable culture conditions. Because of the absence of a cell wall, L-forms are known to be insensitive to β-lactam antibiotics. But reports on the susceptibility of L-forms to other antibiotics that interfere with membrane-anchored steps of cell wall biosynthesis are sparse, conflicting and strongly influenced by strain background and method of L-form generation. We therefore aimed at investigating the response of *B. subtilis* to the presence of cell envelope antibiotics, both with regard to antibiotic resistance and the induction of the known LiaRS- and BceRS-dependent cell envelope stress biosensors [3]. Our results show that *B. subtilis* L-forms are resistant to antibiotics that interfere with the bactoprenol cycle, such as bacitracin and vancomycin, but are hyper-sensitive to nisin and daptomycin, which both affect membrane integrity. Moreover, we established a *lacZ*-based reporter gene assay for L-forms and provide evidence that LiaRS senses its inducers indirectly ("damage-sensing"), while the Bce module presumably detects its inducers directly ("drug-sensing") [4].

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CEV003

Mechanism of substrate recognition of the tRNA-dependent alanyl-phosphatidylglycerol synthase from *Pseudomonas aeruginosa*

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The alanyl-phosphatidylglycerol synthase (A-PGS) from the opportunistic bacterium *Pseudomonas aeruginosa* catalyzes the alanylation of the phospholipid phosphatidylglycerol in a $tRNA^{Ala}$-dependent reaction. When exposed to acidic growth conditions, *P. aeruginosa* synthesizes significant amounts of alanyl-phosphatidylglycerol (A-PG). Furthermore, formation

of A-PG was found responsible for the resistance of *P. aeruginosa* to the CAMP protamine sulphate, the β-lactam antibiotic cefsulodin, the heavy metal ion Cr^{3+} and the osmolyte sodium lactate.
Despite the presence of a large hydrophobic N-terminal transmembrane domain all elements for the catalytic function of the A-PGS are localized in the C-terminal hydrophilic domain ($A\text{-}PGS_{543\text{-}881}$). Using this catalytic fragment an overall of 33 mutant proteins were analyzed *in vitro*. Based on these analyses it was proposed that the enzymatic mechanism proceeds *via* a direct transesterification in an acid-base catalysis of D765. Thereby, the 2' or 3' hydroxyl group of the lipid substrate might nucleophilically attack the α-carbonyl group of Ala-$tRNA^{Ala}$, which is functioning as an activated alanyl-ester substrate.
A-PGS catalysis at the water-lipid interface requires accurate substrate recognition for phosphatidylglycerol and concurrently for the cytosolic co-substrate Ala-$tRNA^{Ala}$. Substrate recognition was analyzed by using aminoacylated microhelices as analogues of the natural tRNA substrate. The enzyme even tolerated mutated versions of this minimal substrate, which indicates that neither the intact tRNA, nor the individual sequence of the acceptor stem is a determinant for substrate recognition. Furthermore, the analysis of derivatives of phosphatidylglycerol indicated that the polar head group of the phospholipid is specifically recognized by the enzyme, whereas modification of an individual fatty acid or even the deletion of a single fatty acid did not abolish A-PG synthesis.
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CEV004

Membrane vesicle formation in *Pseudomonas putida* DOT-T1E as multiple stress response mechanism enhances cell surface hydrophobicity and biofilm formation

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The adaptation of bacteria to a rapid change of environmental conditions is a basic requirement for their survival. Especially the bacterial cell envelope as complex interface to the environment is very sensitive to stress. Therefore, several mechanisms had been evolved with which bacteria respond to the presence of different environmental stresses. Among these mechanisms, the release of outer membrane vesicles (MV) in Gram-negative bacteria has gained research interest especially because of its involvement in pathogenic processes such as that of *Pseudomonas aeruginosa* biofilm formation in cystic fibrosis lungs. In this study we investigated the role of MV formation as an adaptive response of *Pseudomonas putida* DOT-T1E to several stresses and its correlation to biofilm formation. In the presence of long chain alcohols, high NaCl concentrations, EDTA, and after heat shock cells of this strain release MV very rapidly. The formed MV show similar size and charge properties as well as comparable composition in proteins and fatty acids. In addition, this process caused a significant increase in cell surface hydrophobicity and consequently led to an enhanced tendency to form biofilms.
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CEV005

A novel ATP-Driven pathway of glycolipid export for cell envelope formation involving TolC

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Type I secretion systems mediate the transport across the membrane. They are composed of 3 components: inner membrane factor (IMF), membrane fusion protein (MFP) and outer membrane factor (OMF). The IMF is involved in substrate recognition, transport and energy conversion. In case of ABC-exporters anATPbindingcassette, as part of the IMF, provides the energy. The MFP bridges the periplasmic space, connecting the inner with the outer membrane factor. The OMF (TolC and homologues) is a pore-forming membrane-barrel protein that extends into the periplasmic space as an a-helical barrel.
During morphological differentiation to N_2 fixing heterocysts, the filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 forms an extracellular glycolipid layer (HGL). Functioning as O_2 diffusion barrier, this layer is deposited on top of the outer membrane. Mutants defective in any gene of the *devBCA* (*alr3710-3712*) operon, encoding an ABC exporter, are not able to grow on N_2. Although the mutants are not impaired in HGL synthesis, the HGL layer is not present. The *devB* gene encodes a MFP orthologe, *devC* a substrate binding domain of an IMF, and *devA* the respective ATPase (1). A mutant in *alr2887*, encoding a pore-forming TolC-like OMF, is also not able to grow on N_2. It shows the same phenotype like mutants in *devBCA* (2).
We provide evidence that DevBCA and TolC form a type I secretion system required for the direct transport of both HGLs across the gram-negative cell wall. By protein-protein interaction studies (*in vivo* and *in vitro* FA-crosslink, SPR and ITC) we could reveal the kinetic parameters for gating and transport, the stoichiometric relations, the specific binding sites, and indications on a yet unknown mechanism of ATP-driven type I secretion systems. As proposed for the MFP MacA from*E. coli* (3),the homologue DevB needs to connect IMF and OMF as a hexamer. The ATPase activity of the reconstituted DevBCA complex is increased up to seven fold in the presence of purified HGLs. We identified amino acids in DevB that are essential for formation of the hexameric channel and the reaction of the reconstituted complex towards its HGL substrate (4).
Our findings provide a molecular basis for understanding this alternative type of lipid transport system, which represents a novel route for lipids out of the cell.
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CEV006

A non-classical periplasmic protein targeting mechanism

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Most periplasmic proteins carry a hydrophobic N-terminal signal peptide that is required for targeting and export by the Sec machinery. Nevertheless, a few proteins lacking such a peptide have been reported from periplasmic fractions; the mechanism by which these are targeted and exported is currently poorly understood. One of these proteins is the Mn/Fe superoxide dismutase (SodA) of *Rhizobium leguminosarum*, which is also exported to the periplasm of various proteobacteria, including *Escherichia coli*. This protein was used as a model substrate to study the mechanism of non-classical protein targeting. SodA export was inhibited by azide, an inhibitor of SecA ATPase activity. An *E. coli* strain expressing a temperature-sensitive SecA variant also exhibited strongly reduced SodA export, indicating export via a SecA-linked mechanism. By screening various reporter fusion proteins, we showed that the 10 N-terminal amino acid residues of SodA were sufficient to target a reporter protein to the periplasm; further screening of random mutant libraries and directed mutageneses identified a putative targeting signal within this sequence. Although the SecYEG translocon had previously been shown to directly require the binding of classical signal peptides for activation, the identified targeting signal bore no resemblance to any known signal sequence. The targeting and translocation mechanism was therefore further investigated using *in vivo* and *in vitro* translocation assays to identify proteins required for successful targeting and their interactions with the non-classical signal. Our results demonstrate a novel Sec-dependent periplasmic protein targeting mechanism that is independent of a classical signal peptide. Export of SodA to the periplasm is not limited to *Rhizobium*, but was also observed in other proteobacteria. As SodA is a major virulence factor, the secretion and targeting mechanism of this protein may also have significant implications for bacterial pathogenesis.

CEV007

Structural and functional dissection of the Invasin-Intimin family of bacterial adhesins

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Intimin and Invasin are well-characterised virulence factors of enterophathogenic *Escherichia coli* and yersiniae, respectively. These outer membrane proteins belong to a family of proteins whose

extracellular domain is secreted through the outer membrane by a novel autotransport mechanism, termed type Ve secretion [1]. Compared to classical (type Va) autotransporters, Intimin and Invasin have an inverted topology, with the C-terminal passenger being exported through an N-terminal β-barrel pore [2]. In addition, these proteins have an N-terminal periplasmic domain with homology to LysM. We show that the periplasmic domain of Intimin, but not the corresponding, smaller domain of Invasin, binds to peptidoglycan, and that Ca^{2+} ions enhance this binding. Furthermore, the Intimin periplasmic domain mediates dimerisation. The C-terminal passenger domains of Invasin and Intimin contains an array of repeated immunoglobulin (Ig)-like domains [3,4]. We have identified a further Ig domain at the N-terminus of the passenger, which may be involved in passenger export. In addition, we have produced and refolded the β-barrel translocator domain of Invasin for crystallisation trials. The structure of this domain would confirm our topology model and offer insight into this new mechanism of autotransport.

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CEV008

The cell envelope as target of a novel antimicrobial peptide

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Cationic hexapeptide MP196, composed of alternating arginine and tryptophane [3,4], is a promising new antibacterial agent with excellent activity against Gram positive bacteria whereas non-toxic to human cells.
The mechanism of action of this peptide was studied by proteomic investigation of the bacterial stress response, which has been proven to be a useful tool in elucidating antibiotic targets [1,2]. This approach revealed strong similarities of MP196 with potassium ionophore valinomycin as well as cell wall biosynthesis-inhibiting bacitracin. More specifically, we observed strong induction of both membrane stress-induced PspA and cell wall stress-induced LiaH proteins, suggesting a novel or combined cell envelope-related mechanism of action. Further, we investigated the influence of MP196 on membrane integrity and cell wall biosynthesis by several cell-based assays, such as radioactive precursor incorporation, potassium efflux, and membrane potential measurements.
Taken together, our results suggest, that MP196 treatment results in energy and, therefore, nutrient limitation caused by impaired membrane functions.

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CEV009

Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria

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The rod-shaped model gram positive bacterium Bacillus subtilis expresses three isoforms of the prokaryotic actin: MreB, Mbl and MreBH. All three proteins are thought to polymerize into dynamic filamentous helical structures underneath the cell membrane and together with the cell wall (CW) control cell morphogenesis. The prevailing model postulates that membrane-associated MreB filaments spatially organize elongation-specific peptidoglycan-synthesizing complexes along sidewalls.
We have used Total Internal Reflection Fluorescence microscopy (TIRFM) to quantitatively characterize the in vivo distribution and dynamics of fluorescently-labelled MreB proteins and visualize the dynamic relationship between MreB isoforms and CW synthesis proteins in Bacillus subtilis cells. We show that during exponential growth MreB proteins do not form helical structures. Instead, together with other morphogenetic factors (MreC, MreD, PBPH, PBP2a and RodA), they assemble into discrete patches that processively move along peripheral tracks perpendicular to the cell axis. We show with Fluorescence Recovery After Photobleaching (FRAP) experiments that patch motility is not driven by MreB polymerization. Patch motility arrest using CW inhibitors vancomycin and phosphomycin, strongly suggest that the motive force for MreB patches is provided by peptydoglycan (PG) synthesis itself. We also provide evidence that MreB determines rod shape by restricting mobility of elongation complexes.
We propose that 1) CW elongation complexes insert new PG along tracks largely normal to cell long axis, 2) complexes motility is powered by PG polymerization, and 3) MreB acts as a polymeric clamp to restrict the diffusion of CW complexes and allow processive movement in correct orientation.

CEV010

A shortcut pathway to UDP-MurNAc through peptidoglycan recycling in *Pseudomonas*

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In almost all bacteria, the essential cell wall component peptidoglycan is synthesized by a conserved pathway that represents a major target for antibiotics. Synthesis of the soluble cell wall precursor UDP-MurNAc within the cytoplasm involves the essential and highly conserved proteins MurA and MurB. Inhibition of MurA by the antibiotic fosfomycin interferes with peptidoglycan synthesis, causing growth arrest and eventially cell lysis.
Studying peptidoglycan recycling in *Pseudomonas*, we now identified an alternative pathway for UDP-MurNAc synthesis. MurNAc recovered from the own cell wall or scavenged from the environment is directly fed into peptidoglycan synthesis. The pathway involves an anomeric kinase that ATP-dependently phosphorylates MurNAc at the C1 position. Subsequently, an uridyltransferase generates UDP-MurNAc from MurNAc-α-1-phosphate. Mutants in the coding genes accumulated the respective recycling intermediates and showed an increased susceptibility to fosfomycin, indicating the relevance of this pathway for UDP-MurNAc biosynthesis and intrinsic fosfomycin resistance. The pathway is conserved in all *Pseudomonas* strains and many other gram negative bacteria including important pathogens.

CEV011

Identification and *in vitro* analysis of the GatD/MurT enzyme-complex catalyzing lipid II amidation in *S. aureus*

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The peptidoglycan of *Staphylococcus aureus* is characterized by a high degree of crosslinking and almost completely lacks free carboxyl groups, due to amidation of the D-glutamic acid in the stem peptide. Amidation of peptidoglycan has been proposed to play a decisive role in polymerization of cell wall building blocks, correlating with the crosslinking of neighboring peptidoglycan stem peptides. Mutants with a reduced degree of amidation are less viable and show increased susceptibility to methicillin.
We identified the enzymes catalyzing the formation of D-glutamine in position 2 of the stem peptide. We provide biochemical evidence that the reaction is catalyzed by a glutamine amidotransferase-like protein and a Mur ligase homologue, encoded by SA1707 and SA1708, respectively. Both proteins, for which we propose the designation GatD and MurT, are required for amidation and appear to form a physically stable bi-enzyme complex.
To investigate the reaction *in vitro* we purified recombinant GatD and MurT His-tag fusion proteins and their potential substrates, i.e. UDP-MurNAc-pentapeptide, as well as the membrane-bound cell wall precursors lipid I, lipid II and lipid II-Gly_5. *In vitro* amidation occurred with all bactoprenol-bound intermediates, suggesting that in vivo lipid II and/or lipid II-Gly_5 may be substrates for GatD/MurT. Inactivation of the GatD active site abolished lipid II amidation.
Both, murT and gatD are organized in an operon and are essential genes of *S. aureus*. BLAST analysis revealed the presence of homologous transcriptional units in a number of gram-positive pathogens, e.g. *Mycobacterium tuberculosis*, *Streptococcus pneumonia* and *Clostridium perfringens*, all known to have a D-iso-glutamine containing PG. A less negatively charged PG reduces susceptibility towards defensins and may play a general role in innate immune signaling.

CEV012

Synthetic analysis of the apical cell wall synthesis machinery from *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a Gram-positive and non-sporulating soil bacterium with high industrial and medical relevance. Compared to the model organisms *E. coli* or *B. subtilis* for instance, the rod-shaped actinomycete *C. glutamicum* lacks several conserved cell division and shape determining proteins such as the actin homologue MreB, the nucleoid occlusion Noc- and the division site selecting Min system. Morphology and polar elongation is ensured by a machinery composed of the polar determinant DivIVA, the lipid II flippase RodA and several penicillin-binding proteins (PBPs). A second flippase FtsW is part of the divisome and involved in septal growth during division, where it interacts with FtsZ. We recently showed that DivIVA directly interacts with the Par system, thereby providing the polar tethering factor in chromosome segregation. Depletion of *divIVA* as well as deletion of *rodA* both resulted in a coccoid morphology. Using our established syntheticin vivosystem, where *E. coli* cells are used as expression vessels for protein-protein interaction candidates, we provide evidence that DivIVA interacts with RodA, thereby co-localizing it to the cell poles. Individually expressed RodA was distributed randomly around the *E. coli* cell, whereas polar recruitment could only be observed in the presence of DivIVA. To further analyse this interaction, a heterologous FRET system with DivIVA-YFP and RodA-CFP was established. To verify the specificity of the DivIVA and RodA interaction, we included FtsW in ourin vivosystem. However, an interaction of DivIVA and FtsW was not observed. Our data suggest that apical growth in Corynebacteria may depend on recruitment of PBPs upon transpeptidation substrate (lipid II) recognition.

CEV013

Repeating structures of different Gram-positive surface-proteins are essential for the bacterial interaction with human Thrombospondin-1

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Adherence of bacteria to host cells is a multifactorial process and proceeds bacterial infections. The versatile interplay between pathogenic bacteria and its host depend on numerous interactions of bacterial surface structures and host matrix proteins. The matricellular glycoprotein Thrombospondin-1 (TSP-1) is mainly secreted by thrombocytes but also by other human cell types. TSP-1 is a multifunctional, multidomain 420 kDa homotrimer with a wide range of predicted functions in adherence and migration, cell morphology, proliferation and apoptosis as well as in interaction with extracellular proteases. TSP-1 is part of the extracellular matrix and shows binding to different matrix proteins, including fibronectin, fibrinogen, heparin and furthermore to the surface receptors CD36, CD47 and integrin $\alpha_5\beta_1$ (CD49e/CD29). A recent study revealed a new role of TSP-1 for the interplay of different Gram-positive pathogens with host cells (Rennemeier et al., 2007). The TSP-1 was shown to act as a molecular bridge between host cells and Gram-positive bacteria, which facilitated adherence to and invasion into different human epithelial and endothelial cells. Nevertheless, the receptor on the bacterial site as well as on the host site is still unknown. Surface plasmon resonance (SPR) studies with TSP-1 immobilized on CM5 biosensor chip and ligand overlay assays revealed different potential proteinaceous binding partners on the bacterial surface ofStaphylococcus epidermidis,Staphylococcus aureusandStreptococcus pneumoniae. To identify the proteins of interest, 2D-gelelectrophoresis of surface protein fractions was performed and peptides were analyzed by mass spectrometry. Putative candidate proteins fromS. epidermidis andS. pneumoniae were cloned, purified and analyzed for a common binding motif of Gram-positive surface proteins. It turned out that surface-exposed repeats of these proteins are essential for TSP-1-binding activity. The specificity of the TSP-1 interaction with the identified repetitive structures of Gram-positive surface proteins was demonstrated by SPR, ligand overlay assays and competitive inhibition assays. Taken together, this study identified TSP-1 binding motifs in several surface proteins of Gram-positive bacteria involved in recruitment of TSP-1.

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CEV014

The structural basis of staphylococcal cell wall recognition by SH3b domains

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The staphylococcal major autolysin Atl is a bifunctional enzyme consisting of an amidase and a glucosaminidase moiety, separated by internal repeats (R). Processed amidase and glucosaminidase are targeted via the repeat domains (R) to the cell division site. The mechanism behind this precise localization is still unknown. Here, we show by X-ray structural analysis of the repeats that each of the three formerly described repeats consists of two repeats with distinct hydrophobic binding grooves, harboring a GW-(glycin-tryptophan) motif domain that can be blocked by amino acid exchange. We could demonstrate that LTA binding, but not PGN binding depends on the presumptive cell wall binding site. Small-angle X-ray scattering (SAXS) measurement of full-length amidase revealed two inflective linkers between AmiE and R_1 and between R_2 and R_3 that render the amidase highly flexibile.Based on binding studies and structural analysis of the repeat subunits we present a model for targeting amidase to the site of cell division to optimally perform the last step of cell devision, the cell separation.

CEV015

Identification of the trehalose uptake system TusEFGK$_2$ of Corynebacterium gluctamicum and characterization of its role in the biosynthesis of mycolic acids

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Trehalose is a prerequisite for the production of trehalose mycolates (TM), major and structurally important constituents of the cell envelope of Corynebacterineae (2). Mutant strains of *Corynebacterium glutamicum* unable to synthesize trehalose due to the knock-out of the genes of the pathways of trehalose biosynthesis are impaired in growth in minimal medium with sucrose and do not form TM. These effects caused by the abolished trehalose synthesis in *C. glutamicum* ΔotsAΔtreSΔtreY can be compensated by addition of trehalose to the culture broth (2). As hitherto no uptake of trehalose in *C. glutamicum* was detected, it was suggested that trehalose is secreted in a free form followed by subsequent extracellular transfer of mycolyl residues onto the sugar molecule (2). However, the identification of a trehalose uptake system (LpqY-SugABC) in the related species Mycobacterium tuberculosis (1) pointed at the existence of such a system also in *C. glutamicum*. In addition, we observed trehalose utilization by *C. glutamicum* cultivated in minimal medium containing glucose plus trehalose. Taken together these data seriously challenged the above mentioned hypothesis of the free trehalose export for TM synthesis.

We here present the identification and characterization of the trehalose uptake system of *C. glutamicum* as the highly specific ABC transport system TusEFGK$_2$ with an apparent K_m of 0.16 ± 0.02 μM and a V_{max} of 2.5 ± 0.1 nmol/(min * mg cdm). In fact, the substrate binding protein TusE possess only a low identity to the mycobacterial LpqY, yet tryptophan fluorescence-binding assays clearly showed trehalose binding to purified TusE. Deletion of the genomic locus encoding the transporter in *C. glutamicum* Δtus abolished trehalose uptake and utilization.

In addition, we analyzed the effect of trehalose uptake on TM synthesis in the absence of internal trehalose formation and therefore constructed the strain *C. glutamicum* ΔotsAΔtreSΔtreYΔtus. Addition of trehalose to culture broth indeed abolished the growth defects observed for *C. glutamicum* ΔotsAΔtreSΔtreYΔtus and led to the formation of TM. These results indicate that for TM synthesis in *C. glutamicum* ΔotsAΔtreSΔtreYΔtus free trehalose present in the culture supernatant is utilized, which usually has to be export from the cytoplasm, where trehalose synthesis takes place.

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CEV016

Elucidation of the *N*-glycosylation pathway in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*

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Glycosylation is the most dominant form of post translation protein modification. It is proposed that more than 2/3 of the eukaryotic proteins are modified by the attachment of sugar molecules. Due to the common occurrence of glycosylation in eukaryotic proteins, it was long believed that glycosylation is a restricted to this domain of life, however, when in 1976 Mescher and Strominger purified the S-Layer protein from *Halobacterium salinarium*, which contained glycans covalently linked to asparagine residues, questions evoked how *N*-glycosylation occurs in *Bacteria* and *Archaea*.So far the *N*-glycosylation process in crenarchaeota is still uncovered. Here, we will report the first results elucidating the *N*-glycosylation pathway in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*. Deletion studies of selected genes coding for glycosyltransferases mediating the transfer of activated sugar precursors to a lipid carrier and the key enzyme of the glycosylation the oligosaccharyltransferase, showed the essential properties of the *N*-glycosylation process in *Sulfolobus*. Furthermore *S. acidocaldarius* exhibited a unique composition and branched structure of the *N*-linked oligosaccharide, which is linked by a chitobiose core to the S-Layer protein, known to be present in the *N*-glycans of *Eukarya* and so far not found in other *Archaea*.

CEP001

Interaction between histidine kinase and ABC-transporter: new regulatory pathway in antimicrobial peptide resistance modules of *Bacillus subtilis*

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The genome of *Bacillus subtilis* contains three loci (*bceRSAB*, *psdRSAB, yxdJKLM*), which are very similar in gene organization and in sequence, are involved in resistance to various peptide antibiotics. The encoded modules are comprised of a two-component regulatory system (TCS) and an ATP-binding-cassette (ABC) transporter. Both the permease and sensor kinase components show unusual domain architecture: the permeases contain ten transmembrane helices with a large extracellular loop between helices 7 and 8, while the sensor kinases lack any obvious input domain. Strikingly, in the Bce and Psd modules the ABC-transporter and TCS have an absolute and mutual requirement for each other in both sensing of and resistance to their respective antimicrobial compounds, suggesting a novel mode of signal transduction in which the transporter constitutes the actual sensor. Database searches revealed the wide-spread occurrence of such modules among Firmicutes bacteria, and parallel phylogenetic analysis showed that transporters and TCSs have co-evolved. Based on these findings, we hypothesize the formation of a sensory complex between both components, likely involving direct protein-protein interactions between the transport permease and histidine kinase. This is supported by initial results from bacterial two-hybrid assays. To further validate our hypothesis, both the transporter (BceAB) and the histidine kinase (BceS) were expressed heterologously in *E. coli* cytoplasmic membranes and could be purified to high yields. Physical interaction between both protein components will be tested by subsequent *in vitro* interaction and co-purification studies, combined with *in vivo* cross-linking experiments. Taken together, our results show that Bce-type ABC-transporters and TCSs have co-evolved to form self-sufficient detoxification modules against antimicrobial peptides, and suggest a novel signaling mechanism involving formation of a sensory complex between transport permease and sensor kinase.

CEP002

Mapping functional domains of colicin M, a protein toxin from *E. coli*

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Colicin M (Cma), a protein toxin from *E. coli*, is a novel phosphatase concerning sequence, structure and substrate specificity. It is is imported into the periplasm of sensitive cells via a receptor-dependent energy-coupled process. *E. coli* and closely related strains are killey by inhibition of murein biosynthesis; Cma cleaves the phosphate ester bond between the lipid carrier and the murein precursor. This mode of action is unique for Cma. With 271 amino acid residues, it is the smallest of all known colicins. Its fold is unique among colicins and even among all known proteins. The protein forms a compact structure, which makes it difficult to delineate the functional domains which are well-separated in most other colicins [1].

To study these functional domains of Cma, mutants in the various predicted domains were isolated and characterized with special emphasis on the activity domain. The active site is located in a surface-exposed region. Conversion of Asp226 to Glu, Asn, or Ala inactivated Cma. This residue is exposed at the Cma surface and is surrounded by Asp225, Tyr228, Asp229, His235 and Arg236; replacement of each residue with alanine inactivated Cma. We propose that Asp226 directly participates in phosphate ester hydrolysis and that the surrounding residues contribute to the active site. All these residues are strongly conserved in Cma-like proteins of other species.

Moreover, we found that the hydrophobic helix α1, that extends from the compact Cma structure, binds the toxin to the FhuA receptor in the outer membrane and is thereby involved in its uptake [3].

Killing of cells by Cma strictly depends on the periplasmic peptidyl prolyl *cis/trans* isomerase/chaperone FkpA [4]. Because of its compact structure the colicin must unfold during translocation across the outer membrane und refold in the periplasm to be toxic. This is supported by FkpA that presumably assists in refolding by *cis/trans* isomerisation of one or a few prolyl bonds.

To identify the Cma prolyl bonds targeted by FkpA, we replaced the 15 proline residues individually with alanine and found four mutants with reduced activities. P107A displayes 10%, P129A, P176A and P260A show 1% activity. Three of them were not imported, the remaining P176A mutant is structural identical to wild-type Cma which makes it unlikely that the mutation changes the phosphatase active site that is located far from this proline residue. In an *in vitro* peptide assay FkpA isomerized the Cma prolyl bond Phe175-Pro176 at a high rate. These results suppose that this bond is most likely targeted by FkpA in the activation of Cma in the periplasm [4].

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CEP003

Oligomeric structure of the energy transducing ExbB-ExbD-TonB complex

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In *Escherichia coli* and other Gram-negative bacteria energy coupled outer membrane transporters allow the entry of scarce substrates, toxic proteins, and bacterial viruses (phages) into the cells. The required energy is derived from the proton-motive force, which is transduced by the ExbB-ExbD-TonB protein complex from the cytoplasmic membrane. Little is known about the structure and stoichiometry of this complex, which is required to elucidate the mechanisms of energy harvesting at the cytoplasmic membrane and concomitant energy transfer to the outer membrane transporters. We found that C-terminally His6 tagged ExbB and and Strep Tagged ExbD are as functional as wild type. We solubilized an ExbB oligomer and an ExbB-ExbD subcomplex from the cytoplasmic membrane with the help of the detergents decyl and undecyl maltoside. We have purified tagged ExbB oligomer and ExbB-ExbD complex by affinity chromatograph followed by size exclusion chromatography. We have characterized the protein complex in solution by Blue Native PAGE, size exclusion chromatography and small angle X-ray scattering (SAXS). All the methods indicated that there are 4-6 ExbB monomers in the complex. To understand the definite stoichiometry of the complexes we used laser-induced liquid bead ion desorption mass spectrometry (LILBID-MS). At moderate desorption laser energies we determined the oligomeric structure of ExbB to be mainly hexameric ($ExbB_6$), with minor amounts of trimers ($ExbB_3$), dimers ($ExbB_2$), and monomers ($ExbB_1$). Under the same conditions ExbB-ExbD formed a complex consisting of $ExbB_6ExbD_1$, with a minor amount of $ExbB_5ExbD_1$. At higher desorption laser intensities, $ExbB_1$ and $ExbD_1$ and traces of $ExbB_3ExbD_1$, $ExbB_2ExbD_1$, $ExbB_1ExbD_1$, $ExbB_3$, and $ExbB_2$ were observed. Since the $ExbB_6$ complex and the $ExbB_6ExbD_1$ complex remained stable during solubilization and subsequent chromatographic purification on nickel-nitrilotriacetate agarose, Strep-Tactin, and Superdex 200, and during native blue gel electrophoresis, we conclud that $ExbB_6$ and $ExbB_6ExbD_1$ are subcomplexes on which the final complex including TonB is assembled.

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CEP004

Investigation on the subcellular localization of the Gramicidin S synthetase

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Non-ribosomal peptide synthetases (NRPS) enable bacterial and fungal cells to produce a variety of important compounds, like antimicrobial peptides, cytotoxic surfactants or siderophores, as an alternative way to the ribosomal peptide biosynthesis. We investigate the NRPS for Gramicidin S (GS), a cyclic ß-stranded decapeptide, which shows pronounced antimicrobial activity against Gram-positive bacteria, and is also active against Gram-negative bacteria, viruses and fungi. Like all NRPS, the GS synthetase consists of two subunits, GrsA (127 kDa) and GrsB (510 kDa), which are each composed of several domains (A=adenylation, PCP=peptidylcarrier, C=condensation, And TE=thioester).
Despite extensive research on the modular structure of NRPS, little attention has been paid on its subcellular localization in the producing cells. Here, we investigated the localization of GS synthetase inAneurinibacillus migulanus, and Western blot analysis was used to compare cytosolic and membrane fractions of GS-producing and non-producing phenotypes. Immuno-gold electron microscopy was performed with antibodies against the A-domain of GrsA. These combined results show that GS synthetase is localized in the membrane fractions. Based on hydropathy analysis of the A-domain, we then examined its affinity towards different phospholipids. These lipid-protein interaction studies showed an affinity of the GrsA A-domain especially to cardiolipin, which is present inA. migulanusmembranes in high concentration. Our results suggest that it will be possible to optimize the reconstitution of NRPS on solid support materials for the production of peptidesin vitro.

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CEP005

Influence of flotillins on lipid raft dynamics

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Biological membranes are characterized by a high diversity of lipids. Contrary to previous assumptions it could be shown that these lipids are not homogeneously distributed in the membrane but form highly specialized domains, also termed lipid rafts. In these lipid rafts particular proteins are present and can routinely be isolated with these lipid rafts. One subset of these proteins are flotillins. Flotillins normally contain a hairpin loop that tethers the protein to the membrane, accordingly flotillins exhibit a SPFH (stomatin-prohibitin-Flotillin-HflK/C)-domain and a flotillin domain. Furthermore flotillins and other SPFH-domain containing proteins build highly dynamic oligomeric structures. However, the function of flotillins is not yet fully understood but it is generally assumed that they act as scaffolding proteins for lipid rafts. In the living cell it is supposed that highly specialized proteins and lipids are recruited by flotillins to microdomains and form functional complexes. The closest homologue to human flotillin1 can be found in the model organism *Bacillus subtilis*. In previous work we were able to identify several interacting proteins of the flotillin homologue, namely YuaG (FloT). Detergent resistant membranes (DRM) were isolated from a strain expressing SNAP-YuaG. The DRMs were incubated with magnetic beads linked to benzylguanine that covalently binds to the SNAP-tag. Several proteins that are likely interaction partner of YuaG were co-eluted. Strikingly, no crosslinking of these proteins was required for co-elution. One of the identified proteins is the SPFH-domain containing protein YqfA. However, several other proteins were co-eluted with YuaG. Here we show how the identified protein complexes functionally depend on the formation of lipid microdomains.

CEP006

Analysis of the chlamydial translation elongation factor EF-Tu

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The bacterial translation elongation factor EF-Tu is well known to be involveld in prokaryotic protein biosynthesis. EF-Tu from *Escherichia coli* has been shown to polymerize *in vitro* and a recent study provided evidence that the protein serves besides its function in translation another vital role in *Bacillus subtilis*: it contributes to cell shape maintenance, apparently via interaction with the cytoskeleton protein MreB. In rod-shaped bacteria the actin-ortholog MreB is thought to direct incorporation of cell wall material into the side wall. Surprisingly, chlamydiae harbor, despite their spherical shape and the absence of a cell wall, MreB and we recently proved *in vitro* activity for this protein.
Here, we show that EF-Tu from *Chlamydophila pneumoniae* is functional *in vitro*. The purified, strep-tagged protein polymerized in a concentration, pH and ion strength dependent fashion in light scattering and sedimentation assays. Additionally, using co-pelleting assays, we demonstrated that (i) chlamydial EF-Tu interacts with MreB and (ii) the polymerization of MreB is improved in the presence of EF-Tu.
A deeper insight into the functions of EF-Tu and its role in chlamydial cell biology on molecular level will provide valuable information for the design of new anti-chlamydial antibiotics.

CEP007

Investigation of $TatA_d$ oligomerization to a pore complex

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The “twin arginine translocase” (Tat) is a protein export machinery that transports certain folded proteins across the bacterial plasma membrane. The cargo-proteins are targeted to the Tat pathway via an N-terminal signal sequence containing a distinctive twin-arginine motif. The Tat system of *Bacillus subtilis* consists of two essential components, the TatA and TatC proteins, where the transmembrane protein TatA has been suggested to form a protein-conducting channel by self-assembly, but little is known about its oligomeric structure or the translocation mechanism. We have recently discovered a conserved pattern of charged amino acids that are able to form a network of consecutive salt-bridges, and on this basis we proposed a three-dimensional model of the pore-forming complex $TatA_d$. Our hypothesis is that $TatA_d$ could self-assemble via intramolecular and intermolecular salt bridges into tetramers, which can subsequently oligomerize to a pore complex with variable diameter. To test and confirm this model of pore formation, we have produced different di-cysteine mutants to replace the postulated salt-bridges by covalent bridges, which should allow us to distinguish intra- and intermolecular contacts. Further charge mutants $TatA_d$ have been produced to analyze their effect on the oligomerization behavior by SDS-PAGE and Blue-Native PAGE. Single-cysteine side chains have also been introduced into $TatA_d$, to which fluorophores or spin labels can be covalently bound for Fluorescence Correlation Spectroscopy (FCS), Förster Resonance Energy Transfer (FRET) and Electron Spin Resonance (ESR) experiments. The self-assembly of $TatA_d$ monomers into an oligomeric pore complex is being studied using FCS, and distances between $TatA_d$ proteins will be detected by FRET and ESR.

CEP008

S-Layer proteins as platform for nanoscale sensor applications

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In many prokaryotes Surface Layer (S-layer) proteins are the outermost surface of the cell. These self-assembling protein layers have various exciting features. The monomeric proteins are clustered on the cell surface in an entropy-driven process and form paracrystalline highly regular structures. Depending on the organism different arrangements of the protein subunits are possible. They are composed from one to six identical subunits resulting in oblique (p1, p2), square (p4) or hexagonal forms (p3 or p6) of the protein lattice. We investigated proteins with different lattice symmetries for the application in nanostructured sensor chips. Due to the very regular organisation with an ample supply of functional groups (e. g. NH_2^- or SH groups), this lattices should function as the basic building block for a nanosensor. The functionalisation of this sensor is managed by crosslinking of the functional groups to specific receptors for chemical compounds based on Aptamers and a combination with fluorescent dyes. Thus, the sensor could be used for the detection of drugs or other chemicals in fresh- or process water. Here, we present first results on the multimerisation- and binding characteristics of heterologously expressed S-layer fragments as well as coating and coupling experiments for their use in a novel detection system.

CEP009

The cation diffusion facilitator proteins MamB and MamM of *Magnetospirillum gryphiswaldense* are involved in magnetite biomineralization and magnetosome membrane assembly

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Magnetotactic bacteria have the ability to orient along geomagnetic field lines based on the formation of intracellular nanometer-sized, membrane-enclosed magnetic iron minerals, called magnetosomes. The formation of these unique bacterial organelles involves several processes such as cytoplasmic membrane invagination and magnetosome vesicle formation, accumulation of large amounts of iron in the vesicles and crystallization of magnetite. Among the most abundant proteins associated with the magnetosome membrane of *Magnetospirillum gryphiswaldense* are MamB and MamM, which were implicated in magnetosomal iron transport because of their similarity to the cation diffusion facilitator family. Here we demonstrate that MamB and MamM are multifunctional proteins involved in several steps of magnetosome formation. Whereas both proteins are essential for magnetite biomineralization, only deletion of mamB resulted in loss of magnetosome membrane vesicles. MamB stability depended on the presence of MamM by formation of a heterodimer complex. In addition, MamB was found to interact with several other proteins including the PDZ1 domain of MamE, a putative magnetosome associated protease. Whereas any modification of MamB resulted in loss of function, substitution of amino acids within MamM lead to increased formation of polycrystalline instead of single crystals formed in the wild type. A single amino acid substitution within MamM resulted in the formation of crystals consisting of the iron(III) oxide hematite, which coexisted with crystals of the mixed-valence oxide magnetite. Together, the data indicate that MamM and MamB have complex functions and are involved in the control of different key steps of magnetosome formation, which are linked by their direct interaction.

CEP010

Energy conservation in Archaea: the unique way of *Ignicoccus*

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In prokaryotes, only cytoplasmic membranes have been described so far to harbor ATP synthase complexes. The hyperthermophilic, chemolithoautotrophic Crenarchaeon *Ignicoccus hospitalis* (1) is the first organism, which does not follow this rule. The organism exhibits an unusual cell envelope consisting of an inner and an outermost membrane that are separated by a huge inter-membrane compartment (IMC). Recently it has been shown that the ATP synthase and H_2:sulfur oxidoreductase complexes of *I. hospitalis* are located in the outermost membrane (2). As a consequence this membrane is energized by harboring the primary and secondary proton pumps which are necessary for energy conservation within the IMC. As a further characteristic the outermost membrane contains multiple copies of the pore-forming complex Ihomp1, which was proposed to be a prerequisite for the attachment and interaction with *Nanoarchaeum equitans*. Since *I. hospitalis* is the only known host for this organism (3) the localization of all these complexes was investigated in all members of the genus *Ignicoccus*. Immunofluorescence experiments with whole cells showed that the extraordinary localization of the ATP synthase and H_2:sulfur oxidoreductase complex is a common feature of all known members of the genus *Ignicoccus*. Therefore, the outermost membrane of all *Ignicoccus* strains is energized and ATP is generated in the IMC. Further investigations showed that the acetyl-CoA-synthetase which activates acetate to acetyl-CoA by consuming ATP is also associated to the outermost membrane of all *Ignicoccus* members. In contrast, the pore-forming complex Ihomp1 is exclusively found on the cell surface of *I. hospitali*s, supporting the hypothesis of its involvement in the attachment of *N. equitans*.

(1) Paper W. et al. 2007 Int. J. Syst. Evol. Microbiol. 57: 803-808
(2) Kueper U. et al. 2010 PNAS 107: 3152-3156
(3) Jahn U. et al. 2008 J. Bacteriol. 190: 1743-1750
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CEP011

Adsorption kinetics of cell wall components of gram positive bacteria on technical surfaces studied by QCM-D

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In general, the cell wall components of gram-positive bacteria e.g. single lipid bilayer, peptidoglycan, Surface-layer proteins (S-layer) and other biopolymers are well studied. These cell wall components are interesting for several bio-induced technical applications such as biosorptive materials. Although biosorption processes have been intensively investigated, the investigation of metal interaction with biomolecules as well as adsorption processes on substrates on molecular level remains challenging.
In our work we used the quartz crystal microbalance with dissipation monitoring (QCM-D) in order to study the layer formation of cell wall compounds and interaction processes on the nano scale range.
This analytical method allows the detailed detection of array formation of bacterial S-layer proteins and gives a better understanding of the self-assembling processes. S-layer proteins as a part of the outer cell envelope of many eubacteria and archaea form paracrystalline protein lattices in strain depended geometrical structures [1]. Once isolated the proteins exhibit the ability to form these lattices on different kinds of interfaces and possesses equal to the bacteria cells high metal binding capacities. These properties open a wide spectrum of applications e.g. ultrafiltration membranes for organic and inorganic ions and molecules, templates for the synthesis of catalytic nanoparticles and other bio-engineered materials [2, 3].
By performing different experiments with and without modification of technical surfaces with adhesive promoters e.g. polyelectrolytes it is possible to make exact statements regarding coating kinetics, layer stability and interaction with metals. Subsequent atomic force microscopy (AFM) studies enable the imaging of bio nanostructures and reveal complex information of structural properties. Aim of these investigations is the assembly of a simplified biological multilayer based on cell compounds of gram positive bacteria in order to clarify sorption processes in a complex system. The understanding of coating, biological and biological-metal interaction processes is interesting for different technical applications.

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CEP012

Visualization of an S-layer in the anammox bacterium *Kuenenia stuttgartiensis*

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"*Candidatus* Kuenenia stuttgartiensis" is an anaerobic ammonium oxidizing (anammox) bacterium belonging to the order of Brocadiales in the phylum of the *Planctomycetes*. Anammox bacteria are important in nature where they contribute significantly to oceanic nitrogen loss and are applied in wastewater treatment for the removal of ammonium. The cell biology of anammox bacteria is extraordinary; the cells are divided into three membrane-bounded compartments. In addition, the cell wall of *K. stuttgartiensis* does not classify as a typical bacterial cell wall, since it lacks peptidoglycan and does not seem to have a typical outer membrane. The question thus arises how the structural integrity of the cells is maintained. To answer this question the cell wall was studied via freeze etching experiments. Electron micrographs showed the presence of a hexagonal surface layer (S-layer) in the majority of *K. stuttgartiensis* cells. S-layers, crystalline two-dimensional arrays of proteinaceous subunits that make up the outermost layer of many bacterial cell envelopes, have been previously found to have a shape determining function in some bacteria. It is therefore hypothesized that the S-layer could provide structural integrity to the *K. stuttgartiensis* cell. Currently attempts are being made to isolate the S-layer fromK. stuttgartiensiscells to characterize the S-layer and identify the protein (subunits).

CEP013

Role of RodA in *Staphylococcus carnosus* TM300

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Bacteria appear in various shapes like rods, mycetes, cocci, and many more. The cell shape of every bacterium is determined by its cell wall or murein (peptidoglycan), as the murein sacculus provides stability against the internal turgor pressure. For peptidoglycan biosynthesis the interaction of several proteins is required, amongst them are the so called SEDS-family proteins (e.g. RodA, FstW).
SEDS stands for shape, elongation, division, and sporulation, and most of the proteins are essential and can therefore not be deleted. In rod-shaped bacteria like *Bacillus subtilis* or *E. coli* the protein RodA is required for lateral growth of the organism. Yet, a deletion mutant of *rod*A in *E. coli* is known, which is viable in minimal medium as enlarged cocci, albeit with a low growth rate.
In *Staphylococcus carnosus* (*S. carnosus*) there are three genes aligned which encode proteins of the SEDS family, one of them being *rod*A. Since it has never been observed that bacteria of this genus grow in other shapes than cocci, we tried to investigate the role of *rod*A in *S. carnosus*. We were able to completely delete the *rod*A gene and obtain a slow growing but viable mutant. There seems to be no difference in shape or diameter when viewed by electron microscopy. However, the peptidoglycan biosynthesizing machinery is disordered. We could show in a pulse feeding experiment, followed by fluorescent vancomycin labeling that the localization of newly synthesized peptidoglycan is altered compared to the wild type strain. In addition HPLC analysis of digested petidoglycan revealed differences in the muropeptide pattern. Together with our Bacterial-Two-Hybrid experiments, where we obtained an interaction between RodA and all of the four native PBPs of *S. aureus*,these results indicate that RodA indeed plays a role during cell growth of staphylococci, even though these bacteria do not elongate.

CEP014

Penicillin binding protein 2x of *Streptococcus pneumoniae*: the role of different domains for cellular localization

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Penicillin-binding protein 2x (PBP2x) is one of the six PBPs in *S. pneumoniae* involved in late steps of peptidoglycan biosynthesis. PBP2x catalyse a penicillin-sensitive transpeptidation reaction. The PBP2x domain architecture is organized in an N-Terminal PBP dimerization domain, a central transpeptidase domain (TP) and two PASTA (Penicillin binding protein And Ser/Thr protein kinase Associated) domains in its C-terminal region [1]. Mutations in the TP domain of PBP2x that interfere with beta-lactam binding are crucial for the development of high level penicillin-resistance which involves other PBPs as well. The PASTA domains of bacterial Ser/Thr protein kinases exhibit low affinity for beta-lactam antibiotics and are likely to sense peptidoglycan precursors.
As revealed by immunofluorescence techniques localization of PBP2x at the septum has confirmed its role in the division process [2]. However, immunostaining has the disadvantage that cells need to be fixed and have to undergo a damaging cell wall permeabilization treatment. Green fluorescence protein (GFP) fusions can overcome these problems and allow the visualization of fusion proteins in living cells.
To investigate the role of PBP2x during growth and division of *S. pneumoniae* cells, an N-terminal GFP-PBP2x fusion was constructed using a Zn-inducible promoter. Upon induction with Zinc, a GFP-PBP2x signal was observed at the septum in *S. pneumoniae* cells. Furthermore, the native copy of the *pbp2x* gene could be deleted in these cells without affecting cell growth and morphology, showing that GFP-PBP2x is functional. This conditional mutant of *pbp2x* could be grown for five to seven generations in the absence of inducer before depletion of PBP2x was apparent, resulting in distinct phenotypes including significant changes in cell morphology before a complete halt in growth was observed. In order to better understand the role of the various domains of PBP2x for localization at the septum, different mutant constructs in the TP and the C-terminal domain were constructed and characterised. The data show that the PASTA domain is required for localization at the septum.

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CEP015

Inter- and intramycelial DNA-translocation during *Streptomyces* conjugation

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The Gram positive soil bacterium *Streptomyces* transfers DNA in a unique process involving a single plasmid-encoded protein TraB and a double-stranded DNA molecule. TraB proteins encoded by different *Streptomyces* plasmids have a highly specific DNA binding activity and interact only with a specific plasmid region, the *clt* locus, but do not bind to unrelated plasmids. They recognize characteristic 8 bp direct repeats (TRS, TraB Recognition Sequence) via a C-terminal wHTH motif. Exchange of the 13 aa helix H3 of $TraB_{pSVH1}$ against H3 of $TraB_{pIJ101}$ was sufficient to switch specificity of *clt* recognition[1]. Binding of TraB to *clt* is non-covalently and does not involve processsing of the plasmid DNA. In addition to the plasmid localized *clt*, $TraB_{pSVH1}$ also binds to *clt*-like sequences on the chromosome, indicating a chromosome mobilization mechanism independent of plasmid integration.
$TraB_{pSVH1}$ assembles to hexameric ring structures with a central 31 Å channel and forms pores in lipid bilayers. Structure and DNA binding characteristics of TraB indicate that TraB is derived from a FtsK-like ancestor protein suggesting that *Streptomyces* adapted the FtsK/SpoIIIE chromosome segregation system to transfer DNA between two distinct *Streptomyces* cells[1].
In adaptation to the mycelial growth, *Streptomyces* conjugation also involves subsequent spreading of the transferred plasmid within the recipient mycelium. Whereas primary transfer from the donor to the recipient seems to depend on a single protein (TraB), plasmid spreading via septal crosswalls requires five to six plasmid encoded Spd-proteins in addition to TraB. None of the Spd proteins has any similarity to a functionally characterized protein. Bacterial two-hybrid analyses, *in vivo* crosslinking and pulldown assays revealed interactions of TraB and many Spd proteins. Biochemical analyses of purified proteins revealed peptidoglycan-binding activities for TraB, SpdB2, Orf108, SpdA, TraR and DNA-binding activities for TraB, SpdB2, TraR and SpdA. SpdA recognizes a conserved palindromic DNA motif inside the *spdA* coding region[2]. SpdB2 was shown to form pores in planar lipid bilayers.
These data suggest a large DNA translocation complex at the septal crosswalls with TraB acting as the motor protein and SpdB2 probably forming a channel structure.

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CEP016

A highly sensitive enzymatic assay for lytic transglycosylases and their product 1,6-anhydro-N-acetylmuramic acid

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The peptidoglycan (or murein) is a huge, net-shaped glycopeptide macromolecule that surrounds and stabilizes the bacterial cell. It consists of glycan strands composed of two alternating amino sugars N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc). The latter of both is unique to bacteria. During cell growth large amounts of MurNAc-containing fragments (muropeptides) are released from the bacterial cell wall. Fragments carrying a 1,6-anhydroMurNAc moiety at their reducing-end are generated by a special type of muramidases, the lytic transglycosylases (LTs), catalyzing an intramolecular transglycosylation reaction. In E. coli LTs are the main cell wall lytic enzymes, in other bacteria such as B. subtilis the occurance of these enzymes remains unclear.
Since it is difficult to analyze LTs and 1,6-anhydroMurNAc, a novel assay was developed to identify and characterize unknown LTs and determine their specificity. After digestion of purified peptidoglycan with LTs 1,6-anhydroMurNAc was released by total hydrolysis and re-N-acetylation of the samples. In a second step a highly sensitive enzymatic assay was applied which is based on radioactive phosphorylation of 1,6-anhydroMurNAc with anhydroMurNAc-kinase AnmK of E. coli.1 The enzyme specifically converts 1,6-anhydro-MurNAc to MurNAc-6P which is then detectable down to femtomolar amounts by TLC.

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CEP017

Unique wall teichoic acid glycosylation of the borderline Staphylococcus aureus strain PS187 is required for host pathogen interaction

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Staphylococccus aureus is a major human pathogen causing severe diseases including endocarditis, pneumonia and sepsis. Important surface polymers are wall teichoic acids (WTA) known to play a crucial role in a number of processes including host pathogen interaction, biofilm formation, resistance to antimicrobials and phage adsorption. Using a genome sequencing approach S. aureus strain PS187 was found to be a borderline S. aureus isolate sharing almost all classical surface protein adhesins required for host pathogen interaction. Of note one formerly identified nasal colonization factor, WTA, is changed in PS187 to a unique WTA consisting of repetitive units of polyglycerolphosphate (GroP) substituted with D-ala or N-acetylgalatosamine (GalNAc) revised by NMR and renamed as C-type WTA. Based on the genome sequencing approach a novel WTA biosynthesis gene cluster encoding for a unique WTA glycosyltransferase designated as TagN was discovered. Genetic mutants in PS187 lacking C-type WTA and only the GalNAc modification were constructed and used for biochemical analysis. Lectin overlay, WTA PAGE and 1H-NMR analysis clearly demonstrate TagN acts as a WTA GalNAc glycosyltransferase. Based on this C-type WTA glycosylation was found to play a crucial role in phage infection as well as in surviving at high temperatures. Of clinical relevance C-type WTA glycosylation is required for interaction with human epithilial cells indicating PS187 WTA is essential for the colonization process although the WTA structure is different if compared to other S. aureus strains. Hence inhibition of S. aureus WTA glycosylation can be a promising strategy to avoid nasal colonization.

CEP018

Communication and Heterogeneity among *Microcystis* colonies

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Bloom formation of the cyanobacterial genus *Microcystis* represents a worldwide phenomenon reflecting enormous ecological success of these phototrophic bacteria under certain environmental conditions. A sophisticated and diverse formation of intriguing colonial morphotypes reflects heterogeneity as well as genetic plasticity among *Microcystis* cells and is influenced by a comprehensive intercellular communication. In a recently initiated project heterogeneity of *Microcystis* colonies shall be investigated both, from the molecular basis and the physiological effects.
Effects of known impact factors on *Microcystis* colony size like high-light conditions, as well as certain cell surface proteins and other peptides are being systematically monitored and characterized. Insights into the molecular basis of *Microcystis* colony formation shall be gathered by investigating fluorescence labeled *Microcystis* knockout strains, deficient in the production of the cell-cell-interaction affecting proteins and peptides, respectively. Furthermore new sequencing approaches are supposed to clarify genetic conformity or varying genetic composition within cells of *Microcystis* colonies. In addition to the molecular characterization of colonies ecological aspects such as vertical migration properties and enzyme gradients within cell assemblies might give further indications as to the biological benefits ofMicrocystis'sophisticated colony formation.

CEP019

The C-terminal domain confers binding partner specificity to *Bacillus subtilis* DivIVA

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DivIVA proteins are curvature sensitive membrane binding proteins that recruit other proteins to the poles and the division septum. They comprise an N-terminal lipid binding domain fused to less conserved C-terminal coiled coil domains that vary in length and sequence among the different gram positive species. We used bacterial two hybrid analyses to test which part of *B. subtilis* DivIVA is responsible for the interaction to MinJ and RacA. This approach identified short C-terminal truncations of DivIVA that selectively have lost the ability to interact with MinJ and RacA, suggesting that C-terminus of DivIVA is crucial for binding partner recruitment. Complementation experiments of the *B. subtilis* Δ*divIVA* background with chimeric DivIVA proteins that consist of N-terminal stretches of *B. subtilis* DivIVA and corresponding C-terminal portions of DivIVA from *Listeria monocytogenes* furthermore demonstrated that the complete C-terminal coiled coil domain is required for MinJ and RacA binding. Our analyses provide evidence that the C-terminal domain of *B. subtilis* DivIVA is the structural unit that provides the docking site to which MinJ and RacA bind. Fusion of the DivIVA-like lipid binding domain to a less conserved C-terminal protein recruitment module that serves a species-specific cellular function therefore appears to be the unifying architectural feature of DivIVA proteins.

CEP020

Identification of DivIVA interaction partners in *Listeria monocytogenes*

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Cell division, a vital process in all organisms, involves the division of the parent cell into two or more daughter cells, so as to maintain growth and proliferation. DivIVA is a well conserved protein involved in this process in various Gram-positive bacteria, having an N- terminal lipid binding domain (LBD) connected to a C-terminal coiled coil domain (CTD) via a flexible linker. The CTD is postulated to confer diverse morphogenetic functions to DivIVA orthologues in different bacterial species, by allowing it to bind to different interaction partners. Previous work showed a similarity in the phenotype of *divIVA* and *secA2* deletion mutants of *Listeria monocytogenes*, indicating a possible interaction between these two proteins either directly or via some other intermediates. The accessory secretion ATPase SecA2 allows for the translocation of virulence related autolysins and thus contributes to full virulence of *L. monocytogenes*. Bacterial two hybrid assays were used to test for direct interactions between listerial DivIVA, SecA and SecA2. However, these experiments showed only self-interactions but no direct interactions between these proteins, which hinted to the presence of other intermediary interaction partners. Affinity tagged constructs of the respective genes were cloned for the purpose of carrying out affinity pull-down assays using the respective affinity tagged proteins, to identify and characterize these binding partners. This approach will help us to identify so far unknown genes that play a role in SecA2-dependent protein secretion, cell division and virulence pathways of *L. monocytogenes*. Current progress of these experiments would be presented on this poster.

CEP021

Cell wall modifications as a mechanism of antibiotic self-resistance

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Investigations into mechanisms of antibiotic self-resistance in actinomycetes are important to understand the emergence of antibiotic resistance in pathogens and to acquire fundamental knowledge useful for the development of high producer strains by metabolic engineering. An important target for lantibiotics and glycopeptides is the bacterial cell wall. We are currently interested in the study of the self-immunity mechanism in *Microbispora* ATCC PTA-5024 and *Amycolatopsis balhimycina* both synthesizing antibiotics interfering with the bacterial cell wall. *Microbispora* is the producer of NAI-107, the first example of a class I lantibiotic produced by actinomycetes. It inhibits the incorporation of lipid-II in the nascent peptidoglycan by binding to the pyrophosphate moiety. This novel lantibiotic has attracted attention as a potential drug candidate because of its antibacterial profile that cover Gram-positive resistant pathogens like glycopeptide-intermediate *S. aureus* (GISA) and vancomycin-resistant enterococci (VRE) [1]. *A. balhimycina* produces the vancomycin-type glycopeptide balhimycin which binds to the D-Ala-D-Ala ending cell wall precursors. The most common resistance mechanism of bacteria against glycopeptides is to reprogram the murein synthetic machinery resulting in resistant cell wall precursors ending on D-Ala-D-Lac. To understand the self-resistance of the producer and the mode of action of the antibiotic it is important to analyse the cell wall composition of the producer under production and non-production conditions. In contrast to the model organism *Streptomyces coelicolor* M145, both strains *Microbispora* and *A. balhimycina* do not have a monoglycine interbridge but they present a direct linkage between peptide chains. Mature *A. balhimycina* peptidoglycan contains mainly tri- and tetrapeptides and only traces of the D-Ala-D-Ala ending pentapeptides that are binding sites for the antibiotic produced. Both *A. balhimycina* wild type and a non-producing mutant strain synthesize mainly peptidoglycan precursors ending with D-Lac indicating a constitutive synthesis of a resistant cell wall [2]. HPLC-MS analyses of *Microbispora* cell wall precursors reveal a mass peak of 1193.4 Da. This value corresponds to the precursor UDP-

MurNAc-L-Ala-D-Glu-LL-Dap-D-Ala-D-Ala indicating that *Microbispora* protect itself not by synthesizing resistant peptidoglycan.

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CEP022

Interaction and Localisation of the Ser/Thr kinase PknB and the essential two component system YycFG of Staphylococcus aureus

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Prokaryotic signal transduction pathways regulate cellular functions in response to environmental cues and enable bacteria to react immediately to changing conditions like antibiotic stress. Besides two-component regulatory systems (TCS), one-component regulatory systems (OCS) represent one of the most abundant signaling systems in prokaryotes. These OCS include eukaryotic-like serine/threonine kinases (ESTKs) and phosphatases (ESTPs), which are increasingly recognised as important regulators of major processes such as cell wall metabolism and division, virulence/ bacterial pathogenesis and spore formation. One such ESTK/ESTP-couple has recently been identified in Staphylococcus aureus designated PknB/YloO [1]. The extracellular sensor part of the kinase contains three daisy-chained PASTA-domains assumed to be capable of binding peptidoglycan subunits, suggesting that PknB monitors the coordinated assembly of peptidoglycan biosynthesis and cell division. The signal recognised by PknB has not been identified so far.
To further investigate the role of PknB we analysed the interplay with the essential YycFG TCS on the molecular level and show phosphorylation of the response regulator YycF. The YycFG system is involved in the control of peptidoglycan metabolism in S. aureus and both, PknB-GFP and YycG-GFP co-localize at the septum, the site of active cell wall biosynthesis in cocci. This makes an interaction with the cell wall precursor lipid II and subunits thereof, very likely. Determination of the binding parameters to selected lipid II variants, including amidated lipid II and subunits, using quartz crystal microbalance (QCM) biosensor technique will shed light onto the signal recognized by PknB.

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CEP023

New insights into the regulation of the phage shock system in *E. coli*

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The phage shock system is a membrane stress sensor and effector system of *E. coli*, comprising seven genes in three operons. Some of the inducing signals include addition of 10% ethanol, osmotic upshift, severe heat shock, misfolded membrane proteins and disturbance of lipid biogenesis. Stress leads to up-regulation of the systems' genes, namely the gene encoding for the phage shock protein A (PspA), a protein with large coiled-coil domains. Essential parts of the system, especially homologues of PspA, are conserved and widespread among bacteria, archaea and plastids of higher plants, where the protein seems to be responsible for thylakoid membrane formation and organization.
Since PspA production is strongly induced whenever the integrity of the inner membrane is at stake, PspA is thought to exhibit a membrane stabilizing function via direct binding to the inner membrane leaflet. While it is well established that PspA forms multimeric complexes *in vivo* to do so, its mechanism of action is still poorly understood, as well as the regulation of the system itself.
The cellular PspA-level is mainly regulated by a negative feedback-like interaction of PspA with the systems' activator protein PspF. The integral membrane proteins PspB and PspC relay stress signals via direct interaction with PspA, leading to the activation of PspF and therefore higher PspA-levels.
With our new data we provide improved and refined insights into the regulatory aspects of the Psp-regulon, leading to a better understanding of a complex membrane stress system.

CEP025

Recovery of cell wall fragments in *Bacillus subtilis*: Characterisation of D-Glu-mDAP carboxypeptidase and MurNAc-6P etherase

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In *E. coli* and other Gram negative bacteria, the peptidoglycan fragments released during cell growth and division are efficiently reutilised and recycled. In contrast, cell wall recycling in the Gram positive bacterium *B. subtilis* has not been thoroughly studied to date. However, more than 30 autolysins have been identified in this organism that are responsible for cleavage of peptidoglycan and release fragments into the medium during different developmental processes. We are characterising the cell wall turnover products of B. subtilis and are investigating an operon of six genes (*ybbI*, *ybbH*, *ybbF*, *amiE*, *nagZ*, *ybbC*) involved in peptidoglycan recycling. NagZ and AmiE have been functionally characterised recently (Litzinger et al. 2010. J Bacteriol.;192(12):3132-43). NagZ is an Exo-GlcNAc'ase that cleaves the glycosidic bond between non-reducing GlcNAc and MurNAc residues of GlcNAc-MurNAc-peptides (muropeptides), and AmiE subsequently cleaves the MurNAc-peptide bond. Here we report the characterisation of YbbC and YbbI. YbbC was shown to cleave the products of the AmiE reaction, such as L-Ala-D-Glu-mDAP tripeptide. NagZ, AmiE and YbbC, which are secreted, are involved in the sequential digest of muropeptides in the cell wall compartment. The resulting amino acids mDAP, L-Ala-D-Glu dipeptide and amino sugar monomers MurNAc and GlcNAc are imported into the cytoplasm by individual transporters. YbbI is a MurNAc-6P etherase as revealed by the Morgan-Elson assay. MurNAc is phosphorylated to MurNAc-6P by a PTS transporter and then converted to GlcNAc-6P by the cytoplasmic etherase YbbI. Thus, the *ybbIHFEDC* cluster is required for the recycling of cell wall fragments in *B. subtilis*.

CEP026

The role of Lipoprotein STM 3690 in the biogenesis of the trimeric autotransporter adhesin SadA in Salmonella

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Question: Salmonella is a major agent in human food-borne diseases. Among the proteins expressed on the surface of the cells, adhesins, proteins which allow the bacteria to stick to biotic and abiotic surfaces, are key virulence factors.
From the large family of adhesion proteins, the trimeric autotransporter adhesins (TAA) form a distinct subgroup. TAAs are non-fimbrial, non-pilus, homotrimeric adhesins which are widespread among proteobacteria. They have a modular domain structure of extended coiled-coil stretches interspersed with globular domains. The extracellular part of the autotransporter, which can be as large as 100 kDa and above, is transported over the outer membrane of gram-negative bacteria through its own membrane anchor by an unknown mechanism. Several of the autotransporter operons in enterobacteria also contain a small periplasmic lipoprotein of unknown function upstream of the main TAA gene. The location in the operon suggests a supporting role in the folding and export of the passenger domain. The aim of the presented work was to gain insight into the structure of the lipoprotein and to elucidate its function and role in the autotransport of SadA.
Methods: Bioinformatics, Mass spectrometry, Immunofluorescence, FACS, Phage Display, X-Ray Crystallography
Results: Using GCView, a bioinformatics tool for visualizing genomic context for homology search results (1) we could show that the operon of STM 3690 and SadA is conserved in composition and genomic location in Enterobacteria.
We were able to verify that STM 3690 is a periplasmic lipoprotein by subcellular fractionation of bacterial cells expressing the protein. Furthermore we showed the correct lipid modification of the N-Terminus by mass spectrometry.
Cells expressing either SadA and STM 3690 show a higher amount of SadA on the surface compared to cells which express only SadA.
We used Phage Diplay to screen for possible interaction partners of the lipoprotein. This was complemented by pulldowns as well as *in vivo* crosslinks in Salmonella.
Conclusions: Preliminary data suggests a function as chaperone during the export process.

1) Grin I., Linke D. GCView: the genomic context viewer for protein homology searches. Nucleic Acids Res. 2011, 39, W353-W356.

CEP027

Characterisation of the Esx secretion system of *Staphylococcus aureus*

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The Sec-independent Esx (or Type VII) protein secretion pathway is predominantly found in members of the Actinobacteria and Firmicutes. Initially characterised for its role in virulence of *Mycobacterium tuberculosis* [1], Type VII secretion systems have since been associated with a number of diverse processes including conjugative DNA transfer [2] and iron aquisition [3]. In *Staphylococcus aureus*, a Type VII secretion system is present that contributes to virulence by enhancing murine abscess formation and establishing persistent infections [4, 5].
To further assess the role of individual Esx components in protein secretion, unmarked chromosomal deletions of single *esx* genes as well as the whole *esx* operon were created in *S. aureus* strains RN6390 and COL. As the Esx system was found to be involved in various processes not directly related to virulence in Actinobacteria and is furthermore present in a wide range of non-pathogenic Firmicutes bacteria, we also tried to identify alternative pathways affected by deletions of core Esx components.

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CEP028

Identification of a Tat signal peptide-processing protease

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Proteins can be translocated by the twin-arginine translocation (Tat) pathway in a folded conformation. The N-terminal signal peptide of such Tat-substrates is unfolded in solution, even when the remainder of the protein is fully folded. After transport, the signal peptide is usually cleaved off by a signal peptidase. Precursor proteins can Tat-independently interact with membranes via their signal peptide. It has been suggested that this membrane interaction is important for functional transport, as signal peptides can adopt secondary structures at the membrane surface that could trigger the recognition by the Tat system. In our studies on the Tat system of *Escherichia coli*, we noted the generation of a distinct partially processed Tat substrate in the cytoplasm, whereas in the periplasm there was only correctly processed HiPIP detectable. *In vitro* studies revealed that a component of the cytoplasmic membrane catalyzed this specific transport-independent proteolytic turnover. We were able to identify the responsible enzyme as well as the exact cleavage site in the signal peptide and could characterize the requirements for the processing. Interestingly, the cleavage had almost no influence on the translocation efficiency. Together, our data indicate that membrane-interacting Tat substrates encounter proteases that do not abolish transport, at least if the Tat substrate is correctly folded. This observation is discussed in terms of possible roles for a membrane interaction prior to Tat transport.

CEP029

TatA and TatE are membrane-permeabilizing components of the Tat system in *Escherichia coli*

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The twin-arginine translocation (Tat) pathway transports folded proteins across the cytoplasmic membrane of most prokaryotes and the thylakoid membrane of plant plastids. A basic prerequisite for translocation is a stable membrane potential. In *Escherichia coli*, the functional Tat-translocase consists of multiple copies of the proteins TatA, TatB and TatC. There exists a second paralog of TatA, TatE that can functionally substitute TatA. While TatB and TatC form stable complexes in the cytoplasmic membrane that are believed to mediate the specific recognition of Tat substrates, TatA forms separate complexes that only transiently interact with TatBC complexes during translocation. It is believed that TatA complexes somehow facilitate the protein passage. We noted that recombinant TatA strongly affects growth. This effect could be traced back to the N-terminus that forms a trans-membrane domain. Further analyses indicated that this N-terminus alone has the capacity to permeabilize the membrane, which is very unusual for a natural trans-membrane domain and thus strongly suggests a direct function of the TatA N-terminus in the facilitation of the membrane passage. The data support the view that TatA as well as TatE are pore-forming or membrane-weakening constituents of the Tat system.

CEP030

Mode of action of theta-defensins against *Staphylococcus aureus*

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Multicellular organisms defend themselves against infectious microorganisms by producing a wide array of antimicrobial peptides referred to as host defense peptides (HDPs). These evolutionary ancient peptides are important effector molecules of innate immunity and display - in addition to their immunomodulatory functions - potent direct antimicrobial activity against a broad range of pathogens. Generally, HDPs are short (12 to 50 amino acids), positively charged and able to adopt an amphipathic structure.
Among HDPs defensins are an important peptide family characterized by disulfide-stabilized β-sheets as a major structural component. Their mode of action was long thought to result from electrostatic interaction between the cationic peptides and negatively charged microbial membranes, followed by pore-formation or unspecific membrane permeabilization.
Recently, it has been demonstrated that defensin activities can be much more targeted and that fungal (Schneider et al., 2010), invertebrate (Schmitt et al., 2010) and human defensins (Sass et al., 2010; De Leeuw et al., 2010) bind to and sequester the bacterial cell wall building block lipid II, thereby specifically inhibiting cell wall biosynthesis in staphylococci.
Interestingly, the antistaphylococcal mode of action of the cyclic rhesus macaque theta-defensins (RTD-1 and RTD-2) differ from this since RTDs do not affect cell wall biosynthesis. Moreover, the peptides do not compromise the membrane integrity. S. aureus cells treated with RTDs show membranous structures, protrusions of cytoplasmic contents and cell walls peeling off the cell. These morphological changes indicate premature activation of peptidoglycan lytic enzymes involved in cell separation as mechanism of killing.

CEP031

Analyses of the alkaline shock protein 23 (Asp23) of *Staphylococcus aureus*

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With a copy number of about 20,000 molecules per cell, Asp23 is one of the most abundant proteins in *S. aureus*. Asp23 has been characterized as a protein with an apparent molecular mass of 23 kDa that, following an alkaline shock, accumulates in the soluble protein fraction. Moreover, it was shown that the transcription of the *asp23* gene is exclusively regulated by the alternative sigma factor SigB. The function of Asp23, however, has remained elusive. Sequence analysis identified Asp23 as a Pfam DUF322 family member, precluding functional predictions based on its sequence.
Using fluorescence microscopy we found that Asp23 co-localizes with the staphylococcal cell membrane. Interestingly, Asp23 appeared to be excluded from sites of active cell division. Since Asp23 has no recognizable transmembrane spanning domains, we initiated a search for proteins that link Asp23 to the cell membrane. To gain evidence for the function of Asp23, a deletion mutant was constructed and comparative analyses of the wild type and mutant proteome and transcriptome were carried out. These analyses identified a rather small number of differentially regulated transcripts and proteins. Furthermore, using transmission electron microscopy of negatively stained Asp23 protein we showed that it forms large spiral complexes *in vitro*, the formation of which appears to be dependent on the presence of magnesium ions.
In summary, we identified Asp23 as a membrane associated protein in *S. aureus* that forms large, ordered complexes *in vitro*. Identification of the Asp23 function is the subject of ongoing research.

CEP032
Yeast mitochondria as a model system to study the biogenesis of Yersinia Adhesin A (YadA)

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β-barrel proteins are found in the outer membranes of eukaryotic organelles of endosymbiotic origin as well as in the outer membrane of Gram-negative bacteria. Precursors of mitochondrial β-barrel proteins are synthesized in the cytosol and have to be targeted to the organelle. Currently, the signal that assures their specific targeting to mitochondria is poorly defined. To characterize the structural features needed for specific mitochondrial targeting and to test whether a full β-barrel structure is required we expressed in yeast cells the β-barrel domain of the trimeric autotransporter Yersinia Adhesin A (YadA). Trimeric autotransporters are found only in prokaryotes where they are anchored to the outer membrane by a single 12- stranded β-barrel structure to which each monomer is contributing 4 β-strands. Importantly, we found that YadA is solely localized to the mitochondrial outer membrane where it exists in a native trimeric conformation. These findings demonstrate that rather than a linear sequence or a complete β-barrel structure, four β-strands are sufficient for the mitochondria to recognize and assemble β-barrel protein. Remarkably, the evolutionary origin of mitochondria from bacteria enables them to import and assemble even proteins belonging to a class that is absent in eukaryotes.

EMV1-FG
Degradation of organic carbon by microorganisms - do we know the 'rules' and limits?

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The postulate of 'microbial inerrancy' states that for every substance synthesized by organisms there must be at least one type of microorganism able to degrade it. An undegradable biogenic substance would have accumulated in earth's history. This postulate has significantly stimulated biodegradation research. For a long time, many compounds with low chemical reactivity were thought to undergo biodegradation only in the presence of oxygen. However, during the last two decades or so, metabolic types of anaerobic microbes observed in habitats or enriched and isolated in cultures were shown to degrade compounds that formerly were considered recalcitrant under anoxic conditions; a class of such compounds are, for instance, hydrocarbons in gas and oil. Microorganisms degrading chemically unreactive compounds in anoxic habitats are 'confronted' with two challenges, a mechanistic and (often) an energetic one: Bonds may be difficult to activate, and the net energy gain may be very low, respectively. For experimental investigation, also slowness of the processes may present a certain obstacle. Still, on a global scale and over geologically relevant periods, even such slow processes are relevant.

EMV2-FG
Characterising oligotrophic bacterial growth with flow cytometry

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Most natural and engineered aquatic environments comprise a broad diversity of both natural and anthropogenic organic carbon compounds, utilised by an equally broad diversity of indigenous bacterial species. But carbon concentrations are typically low. Total biodegradable organic carbon concentrations below 1 mg/L is common in many lakes, rivers, groundwater and drinking water, and concentrations of individual substrates below 1 μg/L are normal. Bacterial concentrations in such environments are in direct correlation to available substrate concentrations, and typically range from 10^2 to 10^6 cells/mL. These concentrations are several orders of magnitude lower than those usually employed in laboratory based studies, and research is further complicated by the diversity in both the carbon resources and the utilising bacteria. Hence, improved methods for analysing bacterial growth are welcomed. Flow cytometry (FCM) is a method particularly suited for analysis of bacterial growth in these conditions. Firstly, FCM detects all bacteria, irrespective of cultivability. This allows the study of indigenous bacterial communities that do not grow on conventional nutrient media. Secondly, FCM analysis can provide sensitive data on cell concentrations, cell size and nucleic acid content, allowing for detailed information on the organisms in question. Finally, FCM analysis can be automated easily. This provides the opportunity for extensive high resolution analysis of dynamic processes such as bacterial growth. This presentation will discuss the use of FCM in studying (1) indigenous bacterial community growth on natural assimilable organic carbon (AOC), (2) single species (pathogenic bacteria) growth on natural AOC, and (3) single species growth on specific organic carbon compounds.

EMV3-FG
Substrate use of extremely oligotrophic bacteria

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Marine planktonic bacteria live in habitats that are extremely limited in available nutrients, especially the concentration of bioavailable dissolved organic compounds is very low and often close to the detection limit. Therefore, it is difficult to study the substrate use of these bacteria under oligotrophic conditions. Very sensitive methods are needed and it is crucial to keep equipment and medium contamination-free to study the physiology of bacteria proliferating under extremely oligotrophic conditions. The substrate use of *Pseudovibriosp*. strain FO-BEG1 was investigated in artificial and natural oligotrophic seawater on elemental (dissolved organic carbon, DOC and total dissolved nitrogen, TDN) and molecular level. The molecular composition of dissolved organic matter (DOM) was determined by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR-MS) and molecular amino acid analysis. Our data show that the investigated *Pseudovibrio* strain is able to multiply from about 20 cells mL^{-1} to 20,000 cells mL^{-1} in artificial and to 800,000 cells mL^{-1} in natural seawater. DOC concentrations in artificial seawater were < 5 μmol C L^{-1} and 75 μmol C L^{-1} in natural seawater. During growth no significant decrease in DOC and TDN concentrations was detectable. Also N_2 and CO_2 fixation could be ruled out as major nitrogen or carbon source. Interestingly, amino acids were not the primary substrate for growth in both artificial and natural seawater. Among the several thousand compounds detected in seawater, the bacteria were able to use different organic compounds simultaneously, such as organic sulfonates or aminosugars. Most of the metabolized compounds contained nitrogen and thus might serve also as nitrogen source for the bacteria under oligotrophic conditions. Our data demonstrate that many different substrates can be used under extremely oligotrophic conditions at original concentrations. Furthermore, growth in artificial seawater was observed, with DOC concentrations much lower than typically detected in natural oligotrophic seawater.

EMV4-FG
Microbial degradation of organic compounds (natural compounds, xenobiotics, and pesticides) and the formation of soil organic matter and biogenic non-extractable (or bound) residues

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During microbial degradation, carbon from any biodegradable organic compound in soil is partitioned into parent compound, metabolites, non-extractable residues (NER), CO_2, and microbial biomass. This distribution must be known to assess the fate of the compound in soil, e.g. NER from pesticides are considered to consist of adsorbed and sequestered parent compounds or metabolites and thus as hazardous residues. However, they may also partly derive from bacterial biomass, resulting in harmless biogenic residues. In addition, the formation of soil organic matter (SOM) or humic compounds has long been a dominating topic in soil science because the amount and composition of SOM determines soil quality but the processes are still not yet really understood. The so-called humic substances were regarded for a long time as a novel category of cross-linked organic materials. However, the genesis and microbial contribution is still poorly understood. In addition, due to decreasing soil organic matter (SOM) contents all over Europe, a proper management of SOM is needed for maintaining soil fertility and for mitigation of the global increase of the atmospheric CO_2 concentration.
Microbial biomass residues could be identified as a significant source for SOM. We incubated ^{13}C-labelled bacterial cells in an agricultural soil and traced the fate of the ^{13}C label of bacterial biomass in soil by isotopic analysis [1-5]. In the presentation, the mass balance data will be summarized and the microbial biomass and its residues by scanning electron microscopy (SEM) will be visualized. The results indicate that a high percentage of the biomass-derived carbon (in particular from proteins) remains in soil, mainly in the non-living part of SOM after extended incubation. The SEM micrographs only rarely show intact cells. Instead, organic patchy fragments of 200-500 nm size are abundant indicating specific disintegration processes of cell walls. These fragments are associated with all stages of cell envelope decay and fragmentation.

Similar fragments develop on initially clean and sterile in situ microcosms during exposure in groundwater providing clear evidence for their microbial origin. Microbial cell envelope fragments thus contribute significantly to SOM formation. The results provide a simple explanation for the development of the small, nano-scale patchy organic materials observed in soil electron micrographs. They suggest that microstructures of microbial cells and of small plant debris provide the molecular architecture of SOM adsorbed to particle surfaces. This origin and macromolecular architecture of SOM is consistent with most observations on SOM, e.g. the abundance of microbial-derived biomarkers, the low C/N ratio, the water repellency and the stabilisation of microbial biomass [6]. The specific molecular architecture determines carbon mineralisation and balances as well as the fate of pesticides and environmental contaminants. These conclusions were confirmed by studies [7,8] on the biodegradation of isotope labeled 2,4-D and ibuprofen in soil which quantified the contribution of microbial residues to the NER in soil. The amount of label found in biomolecules indicated that virtually all of the NER of the compounds are derived from microbial biomass.

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EMV5-FG
What keeps microorganisms from eating emerging contaminants? - A study on the corrosion inhibitor benzotriazole

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Numerous anthropogenic contaminants are continuously released into freshwater systems where they are typically present in the µg/L range or below. These emerging contaminants might be seen as one of the most widespread environmental problems we are facing today. The corrosion inhibitor benzotriazole (BT) is a high production volume chemical with many industrial and domestic applications which is almost ubiquitously present in the aquatic environment. Although sharing structural similarities with certain biomolecules, neither in sewage sludge nor in oligotrophic freshwater systems microorganisms seem to efficiently degrade BT. For the first time, an aerobic culture could be enriched and maintained that couples biodegradation of BT with growth. Using the enrichment culture, the biodegradation of BT was studied in further detail and inhibitory effects of BT on the degradation of other carbon sources were observed. BT affected biodegradation of other compounds when present at concentrations as low as 20 mg/L. N-methylaniline could be identified as a transformation product of BT based on GC-MS analysis. Although reported to have toxic effects towards microorganisms, N-methylaniline was a less efficient inhibitor of substrate utilization than BT. Our hypothesis is that not the damage to cellular structures or the inhibition of cell functioning in general is responsible for the inhibitory effect of BT but that the compound acts on specific enzymes. In the context of sustainable water quality it is important to come to a better understanding of the inhibitory influence of BT and other emerging contaminants on microbial activities in the environment.

EMV6-FG
Phenoxyacetic acids - what soil microbes can handle ether-linkages in soil?

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4-Chloro-2-methyl-phenoxyacetic acid (MCPA) is one of the best selling herbicides utilized for wheat and lawn control world wide. MCPA is characterized by an ether-bond between a substituted phenol and an acetic acid residue, and subject to aerobic microbial degradation in soil. Previous findings indicated that Beta- and *Gammaproteobacteria* are associated with MCPA degradation in soils. Degradation is initiated by oxygenase-catalyzed cleavage of a glyoxylate residue. Thus, degradation occurs in aerated surface soil and macropores generated by earthworms (i.e., burrow walls). To resolve active MCPA degraders and mine for new oxygenase encoding genes associated with MCPA degradation in bulk and earthworm affected soil, 16S rRNA stable isotope probing (SIP) coupled to structural gene DNA SIP and quantitative PCR was performed. Soil columns were supplemented with [$U^{13}C$]-MCPA at application level concentrations (i.e., 20 µg MCPA g_{DW}^{-1}) in the presence of earthworms. [$U^{12}C$]-MCPA treatments served as controls. MCPA was degraded within 27 days of incubation. Total 16S rRNA analysis revealed 90 active family-level taxa, 33 of which were not affiliated with known families, indicating phylogenetic novelty in bulk soil and drilosphere. 21 and 19 major active taxa occurred in the drilosphere and bulk soil, respectively. 12 of those taxa assimilated MCPA-13C and were affiliated with *Alpha-, Beta-, Gammaproteobacteria*, *Actinobacteria*, and *Firmicutes*. *Sphingomonadaceae* and *Bradyrhizobiaceae* of the Alphaproteobacteria dominated MCPA-assimilating bacteria, indicating that those taxa were major MCPA degraders bulk and earthworm affected soil. In oxic microcosms of bulk soil and burrow wall material supplemented with high concentrations of [U-^{13}C] MCPA (300 µg g_{DW}^{-1}), *Sphingomonadaceae*-related taxa dominated MCPA consumers, while *Betaproteobacteria* (*Burkholderiaceae*-, *Comamonadaceae*-, and *Oxalobacteraceae*-related taxa) dominated MCPA consumers in cast microcosms. Structural gene SIP in such microcosms indicated that MCPA degraders host *tfdA*-like, *cadA* and *r/sdpA* encoding oxygenase genes. Based on 84% protein sequence identity, 49, 6, and 17 operational taxonomic units (OTUs) of were detected in total, including many hitherto unknown genes. Most of the detected genes affiliated with oxygenase genes from *Alphaproteobacteria*. 8, 6, and 4 OTUs of *tfdA*-like, *cadA* and *r/sdpA* genes, respectively, were MCPA-[^{13}C] labeled. Quantitative PCR (qPCR) revealed that copy numbers of such oxygenase genes increased during MCPA degradation in soil microcosms, and the expression of *tfdA*-like and *r/sdpA* genes was stimulated by MCPA, indicating that diverse oxygenase-encoding genes were involved in MCPA degradation. The combined data indicate that (i) *Alphaproteobacteria* rather than *Betaproteobacteria* are major MCPA degrades in certain soils and (ii) new oxygenases are associated with MCPA degradation.

EMV7-FG
A new function for an old yellow enzyme: dearomatizing naphthoyl-CoA reductase, a key enzyme in anaerobic naphthalene degradation

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Polyaromatic hydrocarbons (PAH) are harmful to the environment and human health; they are highly persistent due to the high resonance energy of the ring system and to the low bioavailability. Only little is known about enzymes involved in the anaerobic metabolism of PAHs. The initial activation of naphthalene is considered to proceed by carboxylation yielding 2-naphthoic acid[1,2], which is then activated to 2-naphthoyl-CoA by a specific ligase. Initial evidence was obtained that this key intermediate is dearomatized by reduction[3,4]. Using extracts from the sulphate reducing, naphthalene degrading enrichment culture N47 the time-, protein- and electron donor dependent reduction of 5,6,7,8-tetrahydronaphthoyl-CoA (THNCoA) was demonstrated. This activity (5.1 ± 1.2 nmol min^{-1} mg^{-1}) was sufficiently high for the growth rate of cells; surprisingly it was not oxygen sensitive and not dependent on ATP hydrolysis. Protein purification/characterization including mass spectrometric analysis of tryptic digests revealed that the 2-naphthoyl-CoA reductase (NCR) is a member of the old yellow enzyme (OYE)-family. UV/vis spectra supported the existence of a flavin cofactor and FeS-clusters. The newly identified enzyme represents the prototype of a novel class of aryl-CoA reductases.

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EMP1-FG
Challenging Microbial Infallibility: Investigations on the Biodegradability of Cyclic Peptides

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Diketopiperazines (DKPs) are the smallest possible cyclic peptides composed of two α-amino acids. They are abundant natural compounds produced by a variety of microorganisms as secondary metabolites, e.g. as quorum sensing molecules [1]. Moreover DKPs occur as degradation products e.g. of aminopenicillin antibiotics [2] which are under discussion

as health problem due to the allergenic potential of these exceptional stable compounds.
Although their abundance in nature, little is known about the biodegradation of this substance class and only for few strains hydrolysis of DKPs is reported. In this study we present different approaches to identify potential DKP degrading strains and enzymes, testing eight DKPs synthesized from proteinogenic amino acids and three from non-proteinogenic amino acids (e.g. sarcosine) as substrates:
- Despite peptidase activity against some DKPs has been reported some time ago [3] tested activities could not be confirmed in our lab. Further experiments with additional peptidases indicate peptidase stability for all used DKPs.
- Recently certain cyclic amidases (hydantoinases) have been shown to also cleave dihydropyrimidine derivatives which are structurally related to DKPs [4]. We could demonstrate degradation of different DKPs by three strains exhibiting such cyclic amidase activity. Whether the responsible enzymes are the same is subject of further investigations.
- *Paenibacillus chibensis* (DSM 329) and *Streptomyces flavovirens* (DSM 40062) have been described to hydrolyze the aspartame™ derivative cyclo(L-Asp-L-Phe) [5]. In our studies this activity appeared to be substrate inducible in *S. flavovirens* but not in *P. chibensis*. Moreover we detected the degradation of an additional substrate cyclo(L-Asp-L-Asp) by *P. chibensis* while no other of the tested DKPs was hydrolyzed by one of these strains.
- Two bacterial strains isolated during this study were shown to enantioselectively cleave racemic cyclo(DL-Ala-DL-Ala). We could demonstrate that the cyclo(D-Ala-D-Ala) isomer was not attacked by both strains which were identified as *Microbacterium* sp. and *Paenibacillus* sp. by 16S rDNA sequence analysis.

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EMP2-FG

Ethylbenzene - Isotope fractionation measurements as a tool to characterize aerobic and anaerobic biodegradation

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BTEX compounds (benzene, toluene, ethylbenzene and xylenes) are common pollutants in our environment released from spillings of gasoline. As hydrocarbons are chemically inert compounds they need to be activated to start degradation processes. For long time only molecular oxygen as highly reactive cosubstrate was known to initiate biological decomposition of these compounds. In the last years biochemically completely different mechanisms for initial attack under anoxic conditions were elucidated. Two of them are relevant for ethylbenzene degradation: fumarate addition and oxygen-independent hydroxylation. The better we know which biodegradation process prevails the better it is possible to make reliable predictions for remediation measures.
Here we present isotope fractionation measurements of carbon and hydrogen as a tool to characterize the biodegradation processes of ethylbenzene and a cheap means for monitoring the transformation at contaminated sites. Different reaction mechanisms are reflected by different isotope effects (the result of different reaction rates of molecules containing the light or the heavy isotope). By this way the initial step of various degradation pathways can be differed by determining the single and combined fractionation behaviour of carbon and hydrogen. Investigated ethylbenzene dehydrogenase catalysed reactions by nitrate-reducing test organisms (*Aromatoleum aromaticum*, *Georgfuchsia toluolica* and *Azoarcus* sp.) show a pronounced hydrogen fractionation contrasting to aerobic transformation via hydroxylation of the side-chain or the ring (investigated for *Pseudomonas putida* and an enrichment culture dominated by an *Acidovorax*-related species, respecively. Furthermore masking effects can be excluded by looking at two elements at the same time.
Altogether the newly gained isotopic enrichment factors from various lab cultures will be useful for application at field sites and will complete the picture of isotope effects for BTEX compounds.

EMP3-FG

Chloroethenes in a historical context: From recalcitrance to complete mineralization

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Chloroethenes were identified as common contaminants in groundwater as early as the 1970s (1). Their extensive use as degreasing or dry cleaning solvents and synthetic feed stocks until today has led to groundwater contamination world wide. They are included in the USEPA's list of primary regulated drinking water contaminants (2), because of their toxic and carcinogenic effects on human health.
Common consensus until 1980 was that chlorinated ethenes (Tetrachloroethene (=Perchloroethene, PCE); Trichloroethene (TCE); the three dichloroethenes isomers (cDCE, tDCE, 1,1DCE) and vinyl chloride (VC)) were recalcitrant to biodegradation. This opinion was supported by the fact that these compounds were thought to be only of anthropogenic origin. In addition to that only little importance was assigned to biological processes in groundwater before the 1980s (1).
After several studies on the fate of PCE and TCE in anaerobic groundwater and the accumulation of cDCE or VC as possible transformation products, it was clear at the end of the 1980s that microbial reductive dechlorination can take place in anaerobic, chloroethene contaminated aquifers. From that time on researchers all over the world addressed biological degradation of chloroethenes under different redox-conditions and with a wide range of auxiliary substrates. Today the common opinion is that chloroethenes with higher chlorine content (PCE, TCE) can be degraded more easily under anaerobic conditions serving as electron acceptors and chloroethenes with lower chlorine content (DCE, VC) can be degraded more easily under aerobic conditions serving as electron donors (3).
Here we want to report recent findings on reductive dechlorination, on aerobic metabolism (cometabolic and productive) of lower chlorinated ethenes and on first results indicating that aerobic productive biodegradation of TCE is possible.

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EMP4-FG

Soil microbial communities involved in carbon cycling during leaf litter degradation of annual and perennial plants

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Microbial degradation of plant litter materials provides the primary resources for organic matter formation in soil. The aim of this study was to enlighten the role of bacterial colonisation on leaf litter fragments and to investigate shifts in microbial diversity during leaf litter degradation in the context of carbon cycling.
Therefore, we compared two different litter types: (I)Zea maysas annual and (II)Fagus sylvaticaas perennial model plant. Leafs were sewed into nylon bags and incubated for up to eight (Z. mays) and thirty (F. sylvatica) weeks, respectively, in the soil. The state of degradation was determined by the loss of dry weight. For molecular analyses 16S rRNA genes were detected by two different fingerprinting techniques, terminal restriction fragment length polymorphism (T-RFLP) and enterobacterial repetitive intergenic consensus sequences (ERIC). To get a deeper insight which bacterial communities are involved in litter degradation next generation sequencing using a 454 platform was performed. Additionally, the amount of sugars, amino sugars and phenols was analysed.
First results of the experiment with litter of the annual plant showed a consistent pattern of microbial community shifts. T-RFLP, ERIC and sequencing results reflected concordantly changes of the bacterial community over time. Summarizing, microbial diversity increased during leaf litter degradation and bacterial strains related to carbon cycling such asActinomycetesandMyxococcalescould be identified. Further analyses will reveal how microbial diversity develops on perennial leaf litter. Different results are expected as the litter ofF. sylvaticacontains higher amounts of persistent substances as lignin and celluloses compared toZea mays.

EMP5-FG

Benzotriazole derivatives: biodegradation patterns with three different activated sludge biocenoses

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The compounds benzotriazole (BT), 5-methylbenzotriazole (5-TTri) and 4-methylbenzotriazole (4-TTri) are polar micropollutants widely used as

corrosion inhibitors in dishwashing detergents and in deicing or anti-icing fluids on airplanes. Due to their widespread usage in many applications, their high polarity and therefore good water solubility on one hand and their poor biodegradability on the other, these compounds are found in nearly all aquatic compartments including ground water. For that reason there is urgent need to shed more light on the biological relevance of benzotriazole derivatives. The aim of the present work is to gain insight into selected BT derivatives biodegradation patterns by bench scale tests with three different activated sludge biocoenoses derived from three wastewater treatment systems: WWTP 1 with membrane technology, WWTP 2 equipped with a two-step activated sludge treatment and WWTP 3 with an intermittent nitrification/denitrification regime. After inoculation with 5 g L^{-1} MLSS and 10 mg L^{-1} of BT or else a 10 mg L^{-1} mixture of 4-TTri and 5-TTri (40/60%) the reactors, ranging from 100 to 500 ml in volume, were operated under different nutrient and biomass conditions to evaluate the best setup for aerobically degrading benzotriazole compounds. Biodegradation was shown best for 5-TTri followed by BT and worst for 4-TTri in all reactors regardless which sludge was applied. Concerning the degradation rate over time the sludge from WWTP 1 proved best, followed by WWTP 2 and 3. Also the concentration of nutrients and energy sources such as C- and N-substrates proved to be important. Thus by dosing the benzotriazoles as co-substrates together with an easily utilizable C- and N-source degradation turned out faster compared to reactors fed benzotriazoles as sole C- and/or N-source. In addition to the laboratory experiments environmental water samples were collected to check the influence of WWT retention times on biodegradation. Moreover, benzotriazole concentrations in the receiving rivers were determined. These studies showed in all tested waters benzotriazoles to be found at concentrations ranging from 0.50 to 31.0 μg L^{-1}. WWTPs turned out to constitute one major point source for benzotriazoles into the aquatic environment. Ongoing research is focusing on benzotriazole degrading organisms' or communities' characterization at aerobic and anaerobic conditions and locating of possible intermediates or end-products.

FBV1-FG

No abstract submitted!

FBV2-FG

The carbon depletion response of *Aspergillus niger* during submerged cultivation.

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Background: Filamentous fungi experience carbon limitation in both their natural habitats and biotechnological operations. Compared to nutrient-rich growth conditions, carbon limitation triggers dramatic changes affecting virtually all cellular processes. Liberation of carbon from extra- and intracellular sources fueling fungal self-propagation can be considered as their key response. Comprehensive description of the processes involved and their interactions are important to gain further understanding on a systems-level. Increasing knowledge will be relevant for industrial, medical and fundamental research to improve yields of bioprocesses and develop new antifungal strategies.
Results: This study describes the physiological, morphological and genome-wide transcriptional changes caused by severe carbon limitation during prolonged submerged batch cultivation of the filamentous fungus *Aspergillus niger*. The application of bioreactors allowed for highly reproducible cultivation conditions and monitoring of physiological parameters. We describe the dispersed hyphal morphology at distinct cultivation phases and applied automated image analysis to illustrate the dynamics of cryptically re-growing hyphae. Using the Affymetrix GeneChip platform, we established genome-wide transcriptional profiles for day 1, 3 and 6 of carbon limitation. Compared to exponential growth conditions, roughly 50% (7292) of all genes were differentially expressed during at least one of the starvation time points. To identify major transcriptional trends, we performed enrichment analysis of Gene Ontology, Pfam domain and Kyoto Encyclopedia of Genes and Genomes pathway annotations. Among the predominantly induced processes are autophagy and asexual reproduction. Furthermore, we discuss the transcriptional profiles of enzyme classes, which have been reported to play important roles in aging cultures of filamentous fungi, such as chitinases, glucanases and proteases.
Conclusions: Using an interdisciplinary approach, which combines highly reproducible cultivation conditions with bioinformatics including automated image analysis, genome-wide transcriptional profiling and enrichment analysis, this study provides the first comprehensive analysis of the carbon depletion response in filamentous fungi. The generated data will be fundamental to further improve our understanding of interrelated processes triggered by carbon limitation such as autolysis, proteolysis, cell death, and reproduction.

FBV3-FG

Blood is a very special fluid - the transcriptome of *Aspergillus fumigatus* in response to human blood

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Aspergillus fumigatus is the major cause of Invasive Aspergillosis (IA), a life threatening disease with a mortality rate of 90 to 95 % that affects primarily immunocompromised individuals. A pivotal step linked to severity of this disease is the entry of the fungus into a blood vessel and its dissemination into the blood circuit. Upon entering the blood stream *A. fumigatus* has to adapt to its new environment and to cope with multiple factors. Although transcriptomes of several host infecting fungi have been published recently, knowledge about the adaptation of the *A. fumigatus* transcriptome to blood environment inside the human host is scarce and limits understanding of pathogenesis and *A. fumigatus* dissemination. To gain insight into this part of infection and transcriptional networks involved in this process, we developed an *in vitro* model using human blood mimicking haematogenous dissemination including a time course analysis to elucidate the differences of fungal response at several time points towards blood. This model was used to capture the gene expression that can be found during adaptational processes of the fungus. Samples were analysed by whole genome expression profiling using microarrays followed by gene enrichment analysis and further bioinformatic analysis. Herewith we could identify multiple genes involved in adaptation of *A. fumigatus* to blood such as genes involved in signaling, growth regulation and metabolism. As a first application of our in vitro model we also measured the transcriptional response of *A. fumigatus* to human blood when exposed to the antifungal posaconazole. This gave us the possibility to identify the responses of the fungus when coping with the drug in the environment in which it acts in human treatment. Additionally those data were confirmed using real-time qPCR to support the role of certain genes for the survival of *A. fumigatus* in blood.
This analysis will provide important insights regarding the genes involved in stages of IA and thus may lead the way to new targets for fighting this opportunistic pathogen. The model allows us to test the role of *A. fumigatus* and factors affecting the pathogen in this unique environment.

FBV4-FG

Proteomic profiling of the short-term response of *Aspergillus fumigatus* to hypoxic growth conditions

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Aspergillus fumigatus is an opportunistic airborne pathogen causing systemic infections in immunocompromised patients. This filamentous fungus is an obligate aerobe and requires molecular oxygen for growth. However, during the infection process *A. fumigatus* has to adapt quickly to very low oxygen concentrations when it grows in inflammatory, necrotic tissue. Recently, it was shown that hypoxia is involved in virulence of *A. fumigatus* [1]. In our lab, the metabolic long-term response of this fungus has recently been analyzed by using an oxygen-controlled chemostat [2].
However, little is known about the short-term adaptive mechanisms of *A. fumigatus* to low oxygen concentrations. Therefore, we aimed to investigate the immediate response of *A. fumigatus* after oxygen depletion on the protein level.
A. fumigatus was cultivated as a batch culture in a 3 L bioreactor. After pre-cultivation at 21 % (vol/vol) molecular oxygen concentration, the oxygen supply was shifted to 0.2 % (vol/vol) and several samples were taken during a 24 hour period of hypoxia. Cytosolic protein levels were analyzed by 2D - gel electrophoresis and differentially regulated proteins were identified by MALDI-TOF/TOF-analysis.
Significant changes in the amino acid, carbohydrate and energy metabolism were observed within 24 hours of hypoxia. Glycolytic enzymes and proteins involved in amino acid metabolism were up-regulated. Furthermore, there was an increased production of proteins involved in respiration, electron transport and the general stress response. By contrast, proteins of the pentose phosphate pathway (PPP) and the TCA cycle were down regulated during the short-term response, as well.
Under hypoxic conditions, we determined a strong up-regulation of the alcohol dehydrogenase AlcA which is involved in the utilization of ethanol

[3]. In summary, hypoxia has a strong influence on the metabolic regulation of *A. fumigatus* and the character of the long- and short-term response to hypoxia differs only partly. In future experiments, we will analyze the function of the alcohol dehydrogenase in the adaptation process of *A. fumigatus* to hypoxia in more detail.

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FBV5-FG

Differential analysis of intra- and extra-cellular proteomes of *Verticillium longisporum* during biotrophic and saprophytic growth

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The soil-born, hemibiotrophic plant pathogenic fungus *Verticillium longisporum* causes premature senescence and flowering in oilseed rape (*Brassica napus*), which results in immense agricultural yield reduction. In spite of the significant economical importance of this pathogen, the factors for host specificity are still unknown and the network of virulence factors (effectors) is poorly analyzed. The focus of this study is to identify fungal proteins expressed during plant infection. Therefore we investigated the extra- and intracellular changes of the *V. longisporum* proteome induced by oilseed rape xylem sap (biotrophic model) versus conventional saprophytic growth media. Procedures for the isolation and purification of proteins were optimized for *Verticillium* samples. Protein extracts were separated by one- and two-dimensional gel electrophoresis and peptide samples were analyzed by MALDI-TOF and LC-MSMS. The resulting spectra were searched against peptide data derived of the draft genome sequence of *V. longisporum* 43 we are currently assembling and annotating. Exoproteomes vary to a great extent depending on growth medium, growth phase and light conditions. The identified proteins and their functional categories may represent the different phases of the infection cycle. We identified adhesins and many different groups of carbohydrate-active enzymes like polysaccharide lyases and glycosyl hydrolases, which could be important for penetration and degradation of structurally complex pectin molecules of the plant. Additionally several members of peptidase families were detected, which might be important for proteolysis of host substrates or host defense proteins. Furthermore many small cysteine-rich proteins and necrosis and ethylene-inducing-like proteins (NLP) were identified, which are potential effectors in pathogenicity. Candidate genes and proteins are currently analyzed regarding their importance during plant infection.

FBV6-FG

Regulation of fruiting body formation in *Coprinopsis cinerea*

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Fruiting body formation in the edible dung fungus *Coprinopsis cinerea* is regulated by environmental cues (nutrients, temperature, light, humidity), physiological conditions (mycelial status, age) and genetic control elements (e.g. *A* and *B* mating type genes, *dst1* and *dst2* genes for light receptors). Fruiting body development consists of a series of defined steps occurring in a concerted process over seven days. Following hyphal aggregation, stipe and cap tissues differentiate controlled by light and dark phases. Once light-induced karyogamy takes place in basidia present at the surface of the gills in the cap of mature primordia, stipe elongation and cap expansion start parallel to meiosis in the basidia and subsequent basidiospore formation. All these processes are expected to appoint numerous intracellular as well as extracellular protein functions, many of which might be specific to steps in fruiting. Since the genome of the fungus is available, identification of proteins can now be addressed by large scale proteomic techniques. Stipe and cap fractions from immature fruiting bodies at meiosis are shown to differ from each other, both in the intracellular and the extracellular proteome.

FBV7-FG

The stress-activated protein kinase FgOS-2 is a key regulator in the life cycle of the cereal pathogen *Fusarium graminearum*

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Fusarium graminearumis one of the most destructive pathogens of cereals and a threat to food and feed production worldwide. It is an ascomycetous plant pathogen and the causal agent of Fusarium head blight disease in small grain cereals and of cob rot disease in maize. Infection with *F. graminearum* leads to yield losses and mycotoxin contamination. Zearalenone (ZEA) and deoxynivalenol (DON) are hazardous mycotoxins; the latter is necessary for virulence towards wheat. Deletion mutants of the *F. graminearum* orthologue of the *Saccharomyces cerevisiae* Hog1 stress-activated protein kinase, FgOS-2 (ΔFgOS-2), showed drastically reduced *in planta* DON and ZEA production. However, ΔFgOS-2 produced even more DON than the wild type under *in vitro* conditions, whereas ZEA production was similar to that of the wild type. These deletion strains showed a dramatically reduced pathogenicity towards maize and wheat. We constitutively expressed the fluorescent protein dsRed in the deletion strains and the wild type. Microscopic analysis revealed that ΔFgOS-2 is unable to reach the rachis node at the base of wheat spikelets. During vegetative growth, ΔFgOS-2 strains showed greater resistance against phenylpyrrole and dicarboxymide fungicides. Growth was retarded upon osmotic treatment: the growth rate of mutant colonies on agar plates supplemented with NaCl was reduced but conidia formation remained unchanged. However, the germination of mutant conidia on osmotic media was severely impaired. Germ tubes were swollen and contained multiple nuclei. The deletion mutants completely failed to produce perithecia and ascospores. Furthermore, FgOS-2 also plays a role in reactive oxygen species (ROS)-related signalling. The transcription and activity of fungal catalases is modulated by FgOS-2. Among the genes regulated by FgOS-2 we found a putative calcium-dependent NADPH-oxidase (*nox*C) and the transcriptional regulator of ROS metabolism, *atf1*. The present study describes new aspects of stress-activated protein kinase signalling in *F. graminearum*.

FUV001

Genomics and transcriptomics based on next-generation sequencing techniques to characterize fungal developmental genes

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Next-generation sequencing (NGS) techniques have revolutionized the field of genomics/functional genomics. We have recently sequenced and assembled the genome of the filamentous ascomycete *Sordaria macrospora*, a model organism for fungal development, solely from NGS reads (PLoS Genet 6:e1000891). We are currently applying NGS in two approaches for the identification and characterization of developmental genes. (I) With laser capture microdissection, we can separate protoperithecia from the surrounding hyphae. RNA isolation and amplification from 150 protoperithecia yields enough material for RNA-seq analysis. The resulting data were compared to RNA-seq data from whole mycelial exctracts to characterize the genome-wide spatial distribution of gene expression during sexual development. Additionally, we used the RNA-seq information to improve the predicted *S. macrospora* gene models, and annotated UTRs for more than 50 % of the genes. (II) We sequenced the genomes from three mutants that were generated by conventional mutagenesis, and identified the three causative mutations through bioinformatics analysis. One mutant carries a mutation in the developmental gene *pro41*. The second, a spore color mutant, has a point mutation in a gene that encodes an enzyme of the melanin biosynthesis pathway. In the third mutant, a point mutation in the stop codon of a conserved fungal transcription factor causes the sterility of the mutant. For all three mutants, transformation with a wild-type copy of the affected gene restored the wild-type phenotype. These data show that whole genome-sequencing of mutant strains is a rapid method for the identification of developmental genes.

FUV002

VipA - a novel player in light sensing and development in *Aspergillus nidulans*

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In the filamentous ascomycete *A. nidulans* development and metabolism are strongly regulated by light. In light *A. nidulans* undergoes an asexual life cycle with formation of conidiophores and conidiospores whereas in the dark sexual development with ascospore formation and production of secondary metabolites takes place [1]. For light detection of several wavelengths *A. nidulans* harbors different photosensors like the

phytochrome FphA for red light sensing and the White Collar homologue LreA for blue light detection. A central regulator is the Velvet protein, an FphA interaction partner [2].
Here, we report about a novel Velvet interaction partner, VipA (velvet interacting protein A). VipA is a 334aa protein including a FAR1 domain. FAR1 proteins are well known from plants like *Arabidopsis thaliana* where members of this protein family are involved in phytochrome controlled far-red light responses [3,4]. In *A. nidulans* a *vipA* deletion strain produced only 36% of conidiospores compared to wildtype. This finding points to an activating role of VipA in asexual development. In contrast VeA shows an inhibitory effect [5]. VeA - VipA interaction was shown by yeast-two hybrid analysis and bimolecular fluorescence complementation. The two proteins interact in the nuclei. VipA represents a new element in the regulatory network of spore formation in *A. nidulans*. Detailed analyses on gene regulation through VipA and its relation to other light regulators are on the way.

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FUV003

Alternative splicing in the fungal kingdom

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During gene expression of higher eukaryotes, alternative splicing (AS) can produce various isoforms from one primary transcript. Thus, AS is thought to increase a cell's coding potential from a limited gene inventory. Although AS is common in higher plants and animals, its extent and use in fungi is mostly unknown. We undertook a genome-wide investigation of alternative splicing in 28 fungal species from the three phyla Ascomycota, Basidiomycota and Mucoromycotina, applying current bioinformatics data mining techniques. Our analysis reveals that on average over the investigated fungi, 6.2% of the genes are associated with AS. Cryptococcus neoformans and Coccidioidis immitis show outstanding rates of 18% and 13%, respectively. Intron retention is the predominant AS type in fungi, whereas exon skipping is very rare. The investigated Basidiomycota have on average higher AS rates (8.6%) and more diverse categories of AS affected genes than the Ascomycota (AS rate 7.0%, excluding yeasts). Contrarily, AS is nearly absent in strict yeasts. We hypothesize that AS is rather common in many fungi and could facilitate mycelial and thallic complexity.

FUV004

Transcription factors controlling sporulation in *Magnaporthe oryzae*

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The *Magnaporthe oryzae FLB3* and *FLB4* transcription factor-encoding genes were deleted. Analysis of resultant mutants demonstrated that Flb4p is essential for spore formation and that strains lacking this gene had 'fluffy' colony morphology due to an inability to complete conidiophore formation. Meanwhile Flb3p is required for normal levels of aerial mycelium formation. Using microarray analysis we identified genes dependent on both transcription factors. This analysis revealed that the transcription of several genes encoding proteins previously implicated in sporulation in *Magnaporthe* or in other filamentous fungi are affected by *FLB3* and/or *FLB4* deletion. The transcript changes associated with deletion of *FLB3* and *FLB4* were also reflected phenotypically: the *flb3*-mutant which shows reduced transcription of several secreted lipases and increased transcript abundance for melanin biosynthetic genes has a reduced extracellular lipase activity and increased pigmentation; in contrast the *flb4*-mutant shows reduced transcript abundance for melanin biosynthetic genes and is white.

FUV005

The interaction oft he plant-pathogen *Verticillium longosporum* and its host *Brassica napus* and insights into the evolutionary origin of the fungal hybrid.

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Verticillium longisporum is a soil-borne fungal pathogen of oilseed rape (*Brassica napus*). Infection is initiated by hyphae from germinating microsclerotia which invade the plant vascular system through penetration of the fine roots. We investigated the reaction of the fungus to xylem sap of the host-plant by differential expression of proteins related to reactive oxygen stress [1]. Knockdowns of the catalase-peroxidase of *V. longisporum* were inhibited in the late phase of disease development. The evolutionary origin of the cruciferous fungal pathogen, *V. longisporum* is still a mystery. It is very closely related to both *V. dahliae* and *V. albo-atrum* but possesses some typical characteristics such as long spores, almost double amount of nuclear DNA content and cruciferous host specificity. *V. longisporum* is an example for an early stage of speciation and we show clear evidences for the origin of the fungus. To clarify the hybrid status, we undertook molecular sequence analyses of the internal transcribed spacer (ITS) and intergenic spacer (IGS) regions of rDNA of putative ancestors of *V. longisporum*. In addition a number of other structural genes were analyzed. We found one gene encoding a putative zinc finger transcription factor with two distinct sequences carrying different markers supporting the hybrid origin detection of the fungus. One of these sequences is almost identical to that of *V. dahliae* and the other is highly similar to the sequence of *V. albo-atrum*. Currently we are sequencing *V. longisporum* to determine which rearrangements occurred during and after the hybridization.

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FUV007

The plant pathogenic fungus *Heterobasidion* produces plant hormone-like compounds to elude the plant defense

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The basidiomycete *Heterobasidion annosum* s.l. is a common pathogen of conifers in the northern hemisphere and is responsible for high annual losses in the forest industry [1] by causing the 'annosum root rot' [2]. *H. annosum* s.l. produces a variety of secondary metabolites with different antibiotic activities e.g. fomannosin [3 and 4], fomajorin S [5] and fomannoxin [6]. *H. annosum* s.l. infects its host trees either via exposed woody tissues such as wounds or by fungal growth through root-to-root contacts or grafts with the next tree.
The plants defend themselves against the infection by the necrotrophic pathogen *H. annosum* s.l. [7] by activation of a jasmonic acid / ethylene-dependent signalling pathway, during which the expression of the marker gene *Hel* (encoding a Hevein-like protein) is induced [8]. This signalling pathway can be suppressed by a prior activation of the salicylic acid (SA)-dependent signalling pathway for which the *PR-1* gene (pathogenesis related) is a marker gene [9].
We found two further compounds which are produced by *Heterobasidion* in liquid medium. 5-formylsalicylic acid (5-FSA) is a compound that had previously only been chemically synthesized and 331HaNZ is an unknown compound. 5-FSA and 331HaNZ are structural analogues to salicylic acid. We observed that addition of 5-FSA or 331HaNZ promotes the infection of Norway spruce by *Heterobasidion*. We have also shown that 5-FSA induces the expression of *PR-1* in *Arabidopsis thaliana* and 5-FSA as well as 331HaNZ repress the expression of *Hel* gene after fungal infection. We assume that both compounds repress spruce resistance, resulting in enhanced infection by *Heterobasidion*.

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FUV008

Discovering host specificity candidate genes of *Sporisorium reilianum* by genotyping mixed-variety offspring

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Sporisorium reilianumis a biotrophic plant pathogenic basidiomycete that causes head smut of maize and sorghum. The fungus exists in two varieties with different host specificity. The sorghum variety (*SRS*) is fully virulent on sorghum. *SRS* infection of maize leads to weak symptoms, such as phyllody of the floral parts. The maize variety (*SRZ*) is fully virulent on maize, but does not show symptoms on sorghum inflorescences. Instead, *SRZ* infection of sorghum leads to the formation of red spots containing phytoalexins on leaves.

This different behavior challenged us to find factors responsible for host specificity. We analyze segregants of a mixed-variety infection both phenotypically and genotypically. Approximately 100 offspring of a cross of *SRZxSRS* are tested for virulence on maize and sorghum. Strains that do not lead to disease symptoms on sorghum and those showing full virulence on sorghum are subjected to genotypic analysis by performing species-specific PCRs as well as an NGS approach. Genomic regions stemming from the *SRZ* parent in non-virulent offspring and from the *SRS* parent in virulent offspring are expected to contain candidate genes for host specificity. This way, we identified the beginning of chromosome 7 as one region of interest. This region harbors an SRZ-specific gene (*hsc1*) that, when introduced into *SRS*, was shown to positively contribute to the aggressiveness of the recombinant strains on maize and negatively on sorghum.
This shows that genotyping of mixed-variety offspring is a powerful tool to discover candidate genes involved in host specificity.

FUV006

Induction of manganese peroxidases of wood and leaf-litter colonizing agaricomycetes by olive oil mill residues

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The agroindustrial waste “alpeorujo” (also known as DOR; Dry Olive Residue) is derived from the extraction of olive oil and is produced in large quantities of both solid and liquid wastes in Mediterranean countries.
The residue can be regarded as stimulating natural inductor for oxidoreductases mainly manganese peroxidases (MnP) produced by wood- and litter-decomposing Agaricomycetes. Not only these fungi are able to grow in the presence of nearly toxic amounts of phenol-rich DOR in solid state cultures; but also the increased secretion of oxidative biocatalysts (e.g. up to 1 to 5-fold higher activities for MnP´s of *Bjerkandera adusta*, *Auricularia auricula-judae* and *Agrocybe aegerita*) helps them to detoxify the persistent biopolymeric material. The later was evidenced by an increased shoot and root dry weight of tomato plants grown in the presence of fungal and enzymatically fermented DOR residues indicated a certain detoxification effect (100% within 4 weeks for *A. auricula-judae*, *B. adusta* and *A. aegerita*). The biotransformation of DOR also could be followed by changes in the molecular weight distribution of water-soluble aromatics in the aqueous culture extracts (from 1.5 and 3.5 to 30 kDa) by size exclusion chromatography obviously due to a de-polymerization but also re-polymerization process.
Further analysis of the *de-novo* peptides will allow us to clarify to which MnP type these new representatives belong and which specific MnP genes are activated by DOR residues in the tested fungal organisms.

FUP001

The histone chaperone ASF1 is essential for sexual development in a filamentous fungus

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Ascomycetes develop four major morphological types of fruiting bodies that share a common ancestor, and a set of common core genes most likely controls this process. One way to identify such genes is to search for conserved expression patterns. We analyzed microarray data of *Fusarium graminearum* and *Sordaria macrospora*, identifying 78 genes with similar expression patterns during fruiting body development. One of these genes was *asf1* (anti-silencing function 1), encoding a predicted histone chaperone. *asf1* expression is also upregulated during development in the distantly related ascomycete *Pyronema confluens*. To test whether *asf1* plays a role in fungal development, we generated an *S. macrospora asf1* deletion mutant. The mutant is sterile and can be complemented to fertility by transformation with the wild-type *asf1* and its *P. confluens* homologue. An ASF1-EGFP fusion protein localizes to the nucleus. To test if ASF1 acts as a histone chaperone in *S. macrospora*, we used tandem-affinity purification and mass spectrometry, and identified histones H3 and H4 as putative ASF1 interaction partners. The ASF1-H3 and ASF1-H4 interactions were confirmed by yeast two-hybrid analysis. These data indicate that the *S. macrospora asf1* encodes a functional histone chaperone with a conserved role during fruiting body development.

FUP002

Inhibition of *Verticillium dahliae* in the presence or absence of *Arabidoposis thaliana* by *Streptomyces lividans*

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The ascomycete *Verticillium dahliae* causes worldwide vascular wilt of many field and horticultural plants. During co-cultivation with the soil bacterium *Streptomyces lividans*, the germination of fungal conidia, and the subsequent proliferation are impaired, and fungal conida and microsclerotia arise barely. Upon application of each individual strain to seeds of the model plant *Arabidopsis thaliana*, either the bacterial spores, or the conidia of each fungus germinate at or within the mucilage, including its volcano-shaped structures.The extension of hyphae from each individual strain correlates with the degradation of the pectin-containing mucilage. Proliferating hyphae spread to roots of the emerging seedlings. Plants, which arise in the presence of the *Verticillium* strain, have damaged roots cells, an atrophied stem and root, as well as poorly developed leaves with chlorosis symptoms. *A. thaliana* seeds that have been mixed with the *Verticillium* strain together with *S.lividans*,have preferentially proliferating bacterial hyphae within the mucilage, and at roots of the outgrowing seedlings. As a result, resulting plants have considerably reduced disease-symptoms (1). Using HPLC and LC-MS, we succeeded to purify and characterize *S.lividans* metabolites that provoke the above-outlined effects. Additional results led to deduce that the identified metabolites induce multiple cellular effects, which ultimately impair specific pathways for signal transduction and apoptosis of the fungal plant pathogen (2)
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FUP003

Physiological characterization and synthetic medium development for a model rock-inhabiting black fungus

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Black fungi (a.k.a. black yeasts, meristematic or microcolonial fungi) are the most stress-resistant eukaryotes known to date. These filamentous ascomycetes are able to colonize bare rock surfaces and have evolved passive mechanisms to cope with multiple stresses like high solar irradiation, temperature extremes, low water activity and spare nutrient availability, notably meristematic (isodiametric) growth and incrustation of the cell wall with melanins [1,2]. They are ubiquitous and often involved in primary succession of terrestrial ecosystems by rock weathering and soil formation. Black fungi are therefore an interesting object to study mechanisms of stress resistance (e.g. in astrobiology studies) and are as well used in applied research to prevent material colonization and biodeterioration. Recently, it has been shown that an ancient clade of rock-inhabiting fungi is ancestral to both symbiotic (e.g., lichenized fungi) and pathogenic black fungi [3], which makes them an attractive model to study establishment of symbiotic interactions and evolution of fungal pathogenesis in environmental isolates.
Despite the ubiquity and importance of black fungi in the ecosystem and the interest for research in both basic and applied directions, relatively little is known about their nutritional physiology. Moreover, black fungi are often difficult to cultivate in defined media. Here, we present data produced with the Biolog System [4] to generate a broad physiological profile of the model black fungus *Sarcinomyces petricola* A95 upon cultivation under approximately 1’040 different growth conditions. Knowledge into growth physiology of our model microorganism was used to develop a new *ad hoc* synthetic medium for A95, which we named ASM (for A95-specific medium) [5]. We compared growth of A95 in ASM and in the undefined MEB (2% malt extract broth) and we discuss the obtained data in the light of the oligotrophic character (ability to grow with limited nutrients) of black fungi. We propose that A95 is able to survive in oligotrophic niches by compound re-cycling (cannibalistic mechanism) as observed by maintenance of a low metabolic activity upon absence of primary nutrients (especially sulfur or phosphor sources).
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FUP004

Next-generation genome sequencing, assembly, annotation and analysis of a marine isolate of Scopulariopsis brevicaulis.

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The kingdom fungi constitute the largest branch in the tree of life. However, a very little is known about fungal genomics, although some progress have been made using Sanger sequencing in last two decades. Recently, the Sordaria macrospora genome became available using next-generation sequencing (1). To further explore fungal diversity, we set out to sequence marine isolates of fungi. Here we report the first example, Scopulariopsis brevicaulis, which has previously been known as a common soil saprophyte and has been isolated from a wide variety of substrates. Some species ofScopulariopsisare reported to cause human diseases (2).S. brevicaulisis also known to produce cyclic peptides scopularide A and B (3).
We have established the genomic sequence of a marine isolate ofS. brevicaulisusing three different next-generation sequencing methods namely, roche 454, illumina and ion-torrent. Herein, we present our current results ofS. brevicaulisassembled genome of about 32 Mb size using 726,314, 247,824,350 and 2,556,553 reads from roche 454, illumina and ion-torrent, respectively. We found the contig length is large for roche 454 (935 contigs/N50 - 88 kb) in comparison to contigs of illumina (29330 contigs/N50 - 1.7 kb) and ion-torrent (32008 contigs/N50 - 1.6 kb). Furthermore, we will provide complete annotation of individual assemblies using each sequencing method and also a hybrid assembly achieved using publically and commercially available next-generation sequence assembly and annotation tools. This genome characterization assists fungal biologist to further carry out research with this species, which largely hindered due to unavailability of the genome.

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FUP005

Establishment of an appropriate transformation model for the rock inhabiting fungi *Sarcinomyces petricola* (A95)

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Melanised micro-colonial fungi (MCF) colonize bare rock surfaces in deserts and other arid areas and are unequalled among eukaryotic organisms in their ability to withstand extreme heat, desiccation and UV radiation. These organisms are crucial in the establishment of subaerial rock biofilms and, as such, set the stage for a variety of interactions important for mineral/material stability and rock weathering. MCF are a taxonomically diverse group of ascomycetes and are characterised by simplified stress-protective morphologies including a peculiar compact colonial structure, protective cell walls and multiple secondary metabolic products supporting their stress tolerance - melanins, carotenoids, mycosporines and compatible solutes. A meristematic black yeast species„*Sarcinomyces petricola* (A95), was isolated from a sun exposed marble monument in Athens (Greece). Different methods have been tested to establish a transformation protocol for A95. A common method using the binary Ti vector system of *Agrobacterium tumefaciens* was employed (De Groot et al., 1998). The stress-tolerant morphology of black yeasts, especially their thick cell walls and melanisation complicates the transfer of DNA from *A. tumefaciens* to A95 however. Several methods to circumvent this problem were tested, including DNA transfer by micro-projectile bombardment and chemical weakening of the cell wall by treatment with DMSO. Different protoplasts isolation protocols based on enzymes with chitinase and ß-glucanase activity were also tested. An efficient protocol yielded sufficient protoplasts for transformation with polyethylenglycol. All that remains is to find an appropriate vector system that allows integration of the gene of interest and its translation into the fugal genome.

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FUP006

Influence of microclimatic conditions on fungal diversity in biofilms from the facades of buildings.

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The facades of buildings and their structural elements are colonized by diverse microbes including algae, bacteria and fungi. On older buildings and monuments, these biofilms contribute to the general appearance. Because they cause surface discoloration and material damage, microorganisms that live on the facade of buildings have been the subject of intense interest.
Our research concerns the interaction of subaerial biofilms (SAB) and the underlying substrates. Important components of SAB include melanised micro-colonial fungi (MCF) and phototrophic micro-organisms. Highly melanised MCF are well adapted to extreme environments and thus are stable partners in weathering processes. Currently we are investigating the influence of different microclimatic conditions and seasonal fluctuations on fungal diversity in natural biofilms. A public building in Berlin was chosen for this purpose. Seasonal variations in the composition of the biofilms on the shaded and damp northwest side of the building were compared with those on the sunny and dry southeast side. DGGE analyses based on sequence differences in the 18S rDNA and the ITS rDNA region from different fungi were used to compare the populations. In this way fingerprints of fungal diversity can be generated and compared to other characteristics of biofilm such as chlorophyll contents, spectral properties and other with biofilm partners.

FUP007

Differential analysis of intra- and extra-cellular proteomes of *Verticillium longisporum* during biotrophic and saprophytic growth

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The soil-born, hemibiotrophic plant pathogenic fungus *Verticillium longisporum* causes premature senescence and flowering in oilseed rape (*Brassica napus*), which results in immense agricultural yield reduction. In spite of the significant economical importance of this pathogen, the factors for host specificity are still unknown and the network of virulence factors (effectors) is poorly analyzed. The focus of this study is to identify fungal proteins expressed during plant infection. Therefore we investigated the extra- and intracellular changes of the *V. longisporum* proteome induced by oilseed rape xylem sap (biotrophic model) versus conventional saprophytic growth media. Procedures for the isolation and purification of proteins were optimized for *Verticillium* samples. Protein extracts were separated by one- and two-dimensional gel electrophoresis and peptide samples were analyzed by MALDI-TOF and LC-MSMS. The resulting spectra were searched against peptide data derived of the draft genome sequence of *V. longisporum* 43 we are currently assembling and annotating. Exoproteomes vary to a great extent depending on growth medium, growth phase and light conditions. The identified proteins and their functional categories may represent the different phases of the infection cycle. We identified adhesins and many different groups of carbohydrate-active enzymes like polysaccharide lyases and glycosyl hydrolases, which could be important for penetration and degradation of structurally complex pectin molecules of the plant. Additionally several members of peptidase families were detected, which might be important for proteolysis of host substrates or host defense proteins. Furthermore many small cysteine-rich proteins and necrosis and ethylene-inducing-like proteins (NLP) were identified, which are potential effectors in pathogenicity. Candidate genes and proteins are currently analyzed regarding their importance during plant infection.

FUP008

Asc1p's role in MAP-kinase and cAMP-PKA signaling

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The eukaryotic ribosomal protein Asc1p/RACK1 is required for developmental processes in lower eukaryotes (*S. cerevisiae*) as well as in higher eukaryotes (plants and mammals). However, there is poor knowledge about the protein's exact mode of action and its own post-transcriptional regulation. We could show that *S. cerevisiae* Asc1p controls the abundance of transcription factors in yeast, namely of Ste12p, Phd1p, Tec1p, Rap1p, and Flo8p. This seems to be at least partially due to an Asc1p-dependent translational regulation of the transcription factor mRNAs. We dissect Asc1p's influence on the translation rates of the encoding mRNAs from its putative influence on the stability of the mentioned transcription factors. Tec1p-stability is regulated by the mating response pathway that targets Tec1p for degradation upon phosphorylation through the Fus3p-MAP-kinase. Indeed, the pheromone response pathway is up-regulated in the Δ*asc1* strain. However, pathway inactivation by deletion of the *FUS3* gene did not restore Tec1p levels in the Δ*asc1* strain. Thus, Ascp1 affects Tec1p-abundance via a pheromone-independent mechanism. Shut-off experiments for Tec1p indicate that deletion of *ASC1* has no effect on its stability suggesting an Asc1p-dependent regulation of *TEC1*-mRNA translation. We also analyze whether Asc1p itself is post-translationally modified (e.g. phosphorylated) through MAP-kinase/cAMP-PKA pathways. Modifications of Asc1p could regulate its interaction with other ribosomal proteins or the formation of Asc1p-homodimers [1]. Four phospho-sites of Asc1p are known from high-throughput studies [2,3,4]. Using mass spectrometry we could confirm two of these sites (S166 and T168) and furthermore determined one previously unknown site (T72).

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FUP009

Mode of action of a cell cycle arresting yeast killer toxin

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K28 is a heterodimeric A/B toxin secreted by virally infected killer strains of the yeast *Saccharomyces cerevisiae*. After binding to the cell wall of sensitive yeasts the α/β toxin enters cells via receptor-mediated endocytosis and is retrogradely transported to the cytosol where it dissociates into its subunit components. While β is polyubiquitinated and proteasomaly degraded, the α-subunit enters the nucleus and causes an irreversible cell cycle arrest at the transition from G1 to S phase. K28-treated cells typically arrest with a medium-sized bud, a single nucleus in the mother cell and show a pre-replicative DNA content (1n).
Since other cell cycle arresting killer toxins like zymocin from *Kluyveromyces lactis* or *Pichia acaciae* toxin PaT cause a similar "terminal phenotype", we tested the effect of K28 on *S. cerevisiae* mutants that are resistant against those toxins. Agar diffusion assays showed that deletion of *TRM9* or *ELP3* did not lead to toxin resistance, indicating that the arrest caused by K28 differs from zymocin or PaT induced cell cycle arrest. Interestingly, RNA polymerase II deletion mutants (Δ*rpb4*, Δ*rpb9*) show complete resistance against K28.
To gain deeper insight into the mechanism(s) of how K28α arrests the cell cycle, we further studied the influence of the toxin on transcription of cell cycle and G1-specific genes. Northern blot analyses showed that G1-specific *CLN1* and *CLN2* mRNA levels rapidly decrease after toxin treatment, though it is unclear if this decline is due to a direct effect. Potential toxin targets were identified in a yeast two hybrid screen and verified biochemically by coIP and GST pulldown assays. To confirm that the nucleus represents the compartment where *in vivo* toxicity occurs, we constructed protein fusions between K28α and mRFP and analysed their intracellular localisation.

FUP010

Benzene oxygenation by *Agrocybe aegerita* aromatic peroxygenase (*Aae*APO)

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Agrocybe aegerita aromatic peroxygenase (*Aae*APO) is an extracellular enzyme secreted by the agaric basidiomycete *Agrocybe aegerita*. *Aae*APO hydroxylates the aromatic ring of benzene using hydrogen peroxide as co-substrate. The optimum pH for the reaction is around 7. The reaction proceeds via the primary product benzene oxide which rapidly undergoes aromatization and rearranges to phenol in aqueous solution. Existence of benzene oxide was proved by chemical preparation of this compound and GC/MS and LC-MS analysis. Further oxidation lead to hydroquinone; catechol; *p*-benzoquinone; *o*-benzoquinone as well as 1,2,4-trihydroxybenzene and hydroxy-p-benzoquinone. Using $H_2{}^{18}O_2$ as co-substrate the origin of the oxygen transferred into benzene and phenol was proved to be the peroxide. The use of ascorbic acid as radical scavenger prevented dihydroxy benzenes from exchanging oxygen with water (via quinones) in this investigation. The apparent k_{cat} and the approximated K_M-value for benzene hydroxylation were estimated to 7.9 s^{-1} and 3.6 mM respectively. Benzene oxygenation is first described herein for a heme peroxidase.

FUP011

ER exit of a yeast viral A/B toxin SECrets of K28

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K28 is a virus encoded A/B protein toxin secreted by the yeast *Saccharomyces cerevisiae* that enters susceptible target cells by receptor-mediated endocytosis. After retrograde transport from early endosomes through the secretory pathway, the α/β heterodimeric toxin reaches the cytosol where the cytotoxic α-subunit dissociates from β, subsequently enters the nucleus and causes cell death by blocking DNA synthesis and arresting cells at the G1/S boundary of the cell cycle [1].
Interestingly, K28 retrotranslocation from the ER into the cytosol is independent of ubiquitination and does not require cellular components of the ER-associated protein degradation machinery (ERAD). In contrast, ER exit of a cytotoxic α-variant expressed in the ER lumen depends on ubiquitination, proteasomes and ERAD components, indicating (i) that a most likely masks itself as ERAD substrate and (ii) that ER retrotranslocation mechanistically differs under both scenarios [2]. To elucidate the molecular mechanism(s) of ER-to-cytosol toxin transport in yeast as well as in mammalian cells, the major focus of the present study is to identify cellular components (including the nature of the ER translocation channel) involved in this process. The requirement of proteasomal activity and ubiquitination to drive ER export, and the identification of cellular K28 interaction partners of both, the α/β toxin as well as K28α are being analysedin vitroby using isolated microsomes and IP experiments.

Kindly supported by a grant from the Deutsche Forschungsgemeinschaft (GRK 845).

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FUP012

Adapting yeast as a model to study ricin toxin A uptake and trafficking

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The plant A/B toxin ricin represents a heterodimeric glycoprotein belonging to the family of ribosome inactivating proteins, RIPs. Its toxicity towards eukaryotic cells results from the depurination of 28S rRNA due to the *N*-glycosidic activity of ricin toxin A chain, RTA. Since extention of RTA by a mammalian-specific endoplasmic reticulum (ER) retention signal (KDEL) significantly increases RTA *in vivo* toxicity against mammalian cells, we analyzed the phenotypic effect of RTA carrying the yeast-specific ER retention motif HDEL. Interestingly, such a toxin (RTA^{HDEL}) showed a similar cytotoxic effect on yeast as a corresponding RTA^{KDEL} variant on HeLa cells. Furthermore, we established a powerful yeast bioassay for RTA *in vivo* uptake and trafficking which is based on the measurement of dissolved oxygen in toxin-treated spheroplast cultures of *S. cerevisiae*. We show that yeast spheroplasts are highly sensitive against external applied RTA and further demonstrate that its toxicity is greatly enhanced by replacing the *C*-terminal KDEL motif by HDEL. Based on the RTA resistant phenotype seen in yeast knock-out mutants defective in early steps of endocytosis (Δ*end3*) and/or in RTA depurination activity on 28S rRNA (Δ*rpl12B*) we feel that the yeast-based bioassay described in this study is a powerful tool to dissect intracellular A/B toxin transport from the plasma membrane through the endosomal compartment to the ER.
Furthermore, we established a simple and sensitive fluorescence assay based on thein vivotranslation of a GFP reporter to investigate intracellular RTA trafficking from the endosome to the yeast ER. Our results indicate that both, the mammalian Rab6a homologue Ypt6p, and the yeast syntaxin 5 homologue Sft2p are involved in toxin transport from the endosome to the TGN. In addition, the GARP complex is also important for this trafficking step, whereas defects in the retromer complex did not influence RTA toxicity. Since our results uncovered striking similarities of toxin trafficking between yeast and mammalian cells, we feel that our screening

system represents an attractive alternative to siRNA-based screening systems in mammalian cells.
Kindly supported by a grant from the Deutsche Forschungsgemeinschaft.
B. Becker and M.J. Schmitt (2011). Toxins 7, 834-847.

FUP013

Molecular mechanism of light repression of sexual spore formation in the filamentous fungus *Aspergillus nidulans*

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The filamentous ascomycete *Aspergillus nidulans* is able to perceive light due to a repertoire of light sensors, among which is a pyhtochrome (FphA) and a flavin-containing transcription factor (LreA) (1,2,3). Light triggers many physiological processes and morphogenetic pathways in fungi. For instance in *Aspergillus nidulans*, light induces the development of asexual spores whereas the sexual cycle is preferred in darkness. Whereas light-induction is studied quite well, repression of sexual genes in light has not been studied yet. NosA (numberof sexual spores) and NsdD (never insexualdevelopment) are both important activators of sexual development (4,5). Both proteins localize to the nucleus in all stages of development, which correlates with their function as putative transcription factors and the fact that they both harbor a NLS.
In order to understand the effect of light on these transcription factors, direct interaction between them and light regulator proteins has been studied. Indeed, NosA interacted with FphA in the nucleus as shown by bimolecular fluorescence complementation. This could indicate negative regulation of the NosA activity. In addition, it was found that FphA binds to the promoters of *nosA* and *nsdD* as shown by ChIP (Chromatin-Immunoprecipitation). This suggests transcriptional control of their expression. LreA also bound to the promoter of *nsdD*, but this binding occurred only in light. As the expression of *nsdD* was lower in light than in the dark, LreA appears to repress *nsdD*. As NsdD and NosA are both putative transcription factors, they also activate or repress other genes. CpeA, a catalase-peroxidase, was found to be regulated by NosA. It was shown that NosA binds to the promoter of *cpeA* and that in the *ΔnosA*-strain the expression of CpeA was drastically reduced (4). In addition, a gene with a WSC-domain and a putative FAD-dependent oxidoreductase appeared to be regulated by NsdD.
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FUP014

g1i-diagnosis: development of a strain-specific diagnostic tool for the entomopathogenic fungus *Beauveria brongniartii*

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Mitosporic fungi e.g. of the genus *Beauveria* are of considerable economic and ecological interest as insect biocontrol agents. Strains of the species *Beauveria brongniartii* have been found particularly promising for the control of scarabaeid pests as the European cock-chafer, *Melolontha* sp., and to date several *B. brongniartii* formulations, e.g. "Melocont®", are registered mycoinsecticides. Beyond immediate control efficiencies, parameters as the persistence of fungal spores in the environment, the possible build-up of a residual insecticidal activity, and the long-term impact of biocontrol fungi upon ecosystem biodiversity are of relevance for mycoinsecticide evaluation and registration. Therefore, diagnostic tools for the assessment of these parameters are highly solicited.
In several mitosporic fungi including *Beauveria brongniartii*, nuclear rRNA encoding genes have previously been found interrupted by sequences homologous to self-splicing group 1 introns. We have made use of the presence of these genetic elements to develop a PCR-based approach to strain-specific diagnosis.
Within the framework of research activities aiming towards the development of a *Melolontha* biocontrol strategy based upon endemic fungal isolates from Romania, the Romanian *B. brongniartii* isolate ICDPP#1a was genetically compared to the "Melocont" producer strain. Amplified 18S rRNA encoding sequences from both strains were found to be 100% identical, and strains clustered tightly within the *B. brongniartii* clade of a phylogenetic tree reconstructed from a second, independent marker, namely elongation factor 1 alpha, that is currently the marker of choice for the infra-generic classification of *Beauveria*. However, a difference in the respective 18S rRNA gene exon-intron structures was detected. Based upon this genetic difference, a PCR-based diagnostic tool was developed that renders the two-sided positive discrimination and the differential assessment of the environmental persistence of these biocontrol strains possible.
However, as several conserved intron insertion sites that allow for a considerable number of different exon-intron structures have been identified throughout the 18S and 28S rRNA genes of *Beauveria* and related fungi, g1i-diagnosis clearly holds potential for application beyond this specific context.
Fatu A-C, Fatu V, Andrei A-M, Ciornei C, Lupastean D, Leclerque A (2011) Strain-specific PCR-based diagnosis for *Beauveria brongniartii* biocontrol strains. IOBC/wprs Bulletin 66: 213-216.

FUP015

Will be presented as FUV006!

FUP016

Deneddylation and fungal development

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Deneddylation is the removal of the ubiquitin (Ub)-like protein Nedd8 from cullins. Cullins are subunits of cullin-RING Ub ligases (CRL) which are controlled in their activity and assembly/reassembly by neddylation and deneddylation. The most important eukaryotic deneddylases are the COP9 signalosome (CSN) and the deneddylating enzyme 1 (DEN1). Mammalian Den1 has two functions: an isopeptidase activity removing Nedd8 from cullins and other proteins and an additional linear peptidase activity processing Nedd8 from a precursor protein. Filamentous fungi possess an eight subunit COP9 signalosome (CSN) which is reminiscent to the corresponding plant and vertebrate complex (Busch et al., 2007 PNAS 104: 8089-8094; Braus et al., 2010 Curr Opin Microbiol 13: 672-676). *Aspergillus nidulans* requires CSN function to trigger development in response towards light, and for a coordinated secondary metabolism (Nahlik et al., 2010 Mol Microbiol 78: 964-79). We show here the characterization of the fungal Den1 ortholog DenA. The denA gene encodes a cysteine protease deneddylating enzyme. DenA is required for light control and the asexual fungal development whereas CSN is required for the sexual cycle. Processed Nedd8 is unable to rescue conidia formation suggesting that the lack of the DenA deneddylase isopeptidase activity is responsible for the defect. Yeast-two-hybrid experiments suggest a physical interaction between DenA and CSN which will be further evaluated.

FUP017

Analysis of the F-box protein encoding genes of the opportunistic human pathogen *Aspergillus fumigatus*

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A major virulence factor for the opportunistic human pathogen *Aspergillus fumigatus* is its ability to rapidly adapt to host conditions during infection. The rapid response to environmental changes in the host underlies a well-balanced system of production and degradation of proteins. A highly conserved mechanism for controlled protein degradation is the ubiquitin-proteasome-system. Ubiquitin molecules are attached to the target proteins by the ubiquitin-protein ligase (E3) and therefore polyubiquitnylated proteins are destined for degradation via the 26S-proteasome. The largest group of E3-enzymes is the SCF Cullin1 Ring ligases (CRL), which are multisubunit enzymes. The F-box subunit functions as a substrate adaptor and thus, is responsible for the substrate specificity of the E3 enzyme. In this study we have analyzed the genes, encoding the three F-box proteins Fbx15, Fbx23 and Fbx29 in the opportunistic pathogen *Aspergillus fumigatus*. Deletion of these genes results in growth defects under different stress conditions including H_2O_2 mediated oxidative stress, and increased temperature, which are important parts of the innate immune response. We could further show that the gene for the F-box protein Fbx15 is essantial for virulence of *A. fumigatus* in a murine model. In contrast to this the *fbx15* deletion mutant displays a enhanced production of the immunosupressive mycotoxin, gliotoxin compared to wt and complementation strain. In addition knock-out attemps of *fbx25*, another F-box encoding gene revealed that this is an essantial *fbx*-gene for *A. fumigatus*. Functional GFP-tagged versions of Fbx15 and Fbx25 could be localized in the nucleus suggesting regulatory functions of these F-boxes for certain transcription factors. Future studies aim to identify potential targets of these F-box proteins and their function in stress recognition and response.
This work is supported by the Deutsche Forschungsgemeinschaft, DFG Research Unit 1334.

FUP018

FbFP as an Oxygen-Independent fluorescence reporter in *Saccharomyces cerevisiae* and Candida albicans

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Many microbes colonize anoxic or hypoxic niches and several groups of pathogens attain their virulence by their ability to adapt to these conditions. Although green fluorescent protein (GFP) and its variants are valuable tools for monitoring gene expression and protein localization, their use is limited to aerobic environments, because chromophore synthesis of these reporters requires oxygen. Therefore we established flavin mononucleotide-based oxygen-independent fluorescent proteins (FbFP) as reporters for the apathogenic yeast *Saccharomyces cerevisiae* and the human fungal pathogen *Candida albicans* by expressing the codon-adapted gene encoding CaFbFP under the control of different promoters in both fungi (*Eukaryot. Cell* 8:913-915, 2009). Synthesis of CaFbFP was demonstrated in *S. cerevisiae* and *C. albicans* cells by immunoblotting and fluorescence was detected under both normoxic and hypoxic conditions in the cytoplasm of cells. To examine the use of FbFP as a reporter in other cell compartments we attempted to achieve cell wall localization of FbFP in *S. cerevisiae* by generating fusions to the cell wall protein Aga2. Fluorescence analyses and immunodetection indicated the localization and fluorescence of the FbFP fusion on the yeast cell surface. The ability of FbFP to fluoresce in yeast nuclei was investigated by fusion of CaFbFP to the histone H2B of *S. cerevisiae* and *C. albicans*, respectively. Fluorescence analyses of *S. cerevisiae* cells showed a clear FbFP-mediated fluorescence signal in the nuclei.

FUP019

Screening of white rot fungi from Belarus for novel dye bleaching enzymes

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The textile industry is an industrial branch with great relevance for the environment. During the textile dying process 30 to 40% of dyes do not bind to the cotton fibres and remain in the waste water. The costs for cleaning this waste water with different physical, chemical, electrochemical or biological methods are high. For bleaching of non-bound dyes in the waste water and bleaching of cotton, the use of enzymes from white rot fungi could be an environmentally friendly and also less cost intensive alternative.
We performed a screening for dye decolourisation by novel white rot fungi from National Park Belaweschskaja Puschtscha Belarus. Because of the similar phenolic structure of lignin and industrial dyes, white rot fungi can degrade many of these dyes. The most effective enzymes for this application are laccases and peroxidases. 17 different fungal mixed cultures were investigated for their ability to bleach 40 dyes of yellow, orange, red, blue, and black colour with different chemical structures used in technical textile dying processes. Bleaching experiments were carried out on agar plates and in liquid cultures. From mixed cultures with high decolourisation rates we isolated pure fungal samples for identification.
Whereas many fungi could degrade blue and black dyes, only a few of them could also decolourise yellow, orange, and red ones. Especially the decolourisation of yellow dyes is a problem in many bleaching processes and often a yellow colour remains even after the successful decolourisation of blue or black dyes. Two of our new fungi aMucor hiemalis sp. silvaticusand aMortierella verticillatasp. are able to decolourise yellow dyes very effectively. The main enzymes from some of the fungi with very high bleaching activity were detected and partly purified. We determined laccase, manganese- and ligninperoxidase activities and performed first chromatographic purification steps for these enzymes. Thus we found new enzymes with interesting properties for the use in industrial bleaching processes.

FUP020

Alternative splicing in fungal aldo-keto reductases

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Aldo-keto reductases (AKRs) are characterized by a common 3D-fold, the $(\alpha\beta)_8$-barrel motif and a broad substrate specificity [1]. They are NAD(P)H-dependent andrecognize broad categories of carbonyl-containing substrates, e.g. aldehydes, ketones, monosaccharides, and steroids. The cofactor binding site for NAD(P)H is highly conserved. AKRs encompass a superfamily comprising approx. 120 members in 14 protein families scattering through prokaryotes, plants, animals and fungi. In former studies (manuscript in prep.), we reconstructed the evolution of fungal AKRs using distance, maximum parsimony, maximum likelihood and Bayesian analyses. We are able to recognize different subgroups and paralogs and discovered different alleles. In order to understand the evolution of these different alleles we predicted the possible occurrence of alternative splicing (AS). We applied the bioinformatics tool NetAspGene ([2], http://www.cbs.dtu.dk/services/NetAspGene/), which was originally trained for genes of the ascomycete *Emericella nidulans* (anamorph: *Aspergillus nidulans*), to AKRs from zygomycetes. Known splice sites could be recovered. The prediction of additional splice sites with high confidence scores ranging typically between 0.85 and 1.00 actually suggests a diversification through AS. The impact of AS in the evolution of fungal AKRs are discussed.

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FUP021

Engineering the citric/isocitric acid overproduction by the yeast *Yarrowia lipolytica*

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Functionalized carboxylic acids are highly versatile chemical species with a wide range of applications (e.g. as co-polymers, building blocks, acidulants). Therefore they are of special interest as biotechnologically available targets. The yeast *Yarrowia lipolytica* secretes high amounts of various organic acids, like citric acid (CA) and isocitric acid (ICA) under several conditions of growth limitation from an excess of carbon source. Depending on the carbon source used, strains of *Y. lipolytica* produce a mixture of CA and ICA in a characteristic ratio. On carbohydrates and glycerol, wild-type strains show a CA/ICA ratio of 90:10, and on sunflower oil and n-alkanes of 60:40. To examine, whether this CA/ICA product ratio can be influenced, isocitrate lyase (*ICL1*), aconitase (*ACO1*) or isocitrate dehydrogenase (*IDP1*) overexpressing strains were constructed containing multiple copies of these genes, respectively. Additionally, *ICL1* disrupted strains were tested. In the *ICL1* overexpressing strains the part of ICA on the whole product (CA + ICA) decreased to 3-7% on all tested carbon sources [1]. In contrast, the *ACO1* and interestingly also the *IDP1* overexpression resulted in a shift of the product pattern in direction of ICA [2]. On carbohydrates the ICA proportion increased from 10-12% to 14-15%, on sunflower oil even from 35-45% to 65-72% of total acid produced. The loss of the isocitrate lyase activity in the *icl1*-defective strains had a comparable effect on the CA/ICA ratio like the *ACO1* overexpression. On glucose and glycerol the ICA proportion was 2-5% higher compared to the wild-type strain. Thus, using wild-type or engineered *Y. lipolytica* strains the enantiomerically pure form of isocitric acid, currently available as a speciality compound, can be produced now in large amounts and used as a building block for organic synthesis [3].

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FUP022

A molecular tool for transposon-mediated mutagenesis in *Aspergillus species*

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Transposons are ubiquitous genetic elements present in the genomes of all living cells. Among the different types of transposable elements cut-and paste transposons are particularly useful for development of transposon-based mutagenesis systems. We previously have characterized transposable elements in two filamentous fungi,AspergillusnigerandPenicillium chrysogenum(ref. 1), thereby identifiying transposonVaderas an active element inA.niger Upon selection for chlorate resistantA.nigercolonies, oneVadercopy was found integrated in thenirAgene. As this copy apparently contained all necessary sequence information for beingtrans-activated it was used for vector development and fungal transformation (ref. 2).
We observed a Vaderexcision frequency of about 1 in $2.2x10^5$A.niger spores. All colonies analyzed exhibited an excision event on the DNA level andVader footprints were found. Employing thermal asymmetric interlaced-PCR the reintegration sites of 21 independent excision events were determined. All reintegration events occurred within or very close to genes. Thus,Vader appears to be a useful tool for transposon mutagenesis inA.niger (ref. 2).

We then set out to analyze the activity ofVaderinA. nidulans. TheVaderelement on its does not exhibit any activity inA. nidulans. This was anticipated asVaderlacks the appropriate transposase gene. A new vector including the transposase gene was established and inserted the vector at theniaD locus. Transformants are currently being analyzed for activity ofVader.

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FUP023

Engineering the α-ketoglutarate overproduction by the yeast *Yarrowia lipolytica*

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One of the most prominent features of the non-conventional yeast *Yarrowia lipolytica* is the secretion of high amounts (up to about 200 g/L) of various organic acids, like α-ketoglutaric (KGA), pyruvic (PYR) or citric/isocitric (CA/ICA) acids under special culture conditions. Typically, an excess of carbon source and simultaneous growth limitation by different factors, e.g. thiamine (KGA and PYR) or nitrogen exhaustion (CA andICA) result in overproduction of organic acids. The amount and kind of produced organic acids can be affected by changes of activities of involved enzymes [1-3]. The aim of this study was to improve the KGA overproduction by *Y. lipolytica* from renewable substrates (e.g. glycerol and raw glycerol) and to examine whether the amount of the secreted minor products pyruvate, fumarate and malate can be influenced by a gene-dose dependent overexpression of enzymes of the tricarboxylic acid cycle and of gluconeogenesis. We show that a gene-dose dependent overexpression of the genes encoding isocitrate dehydrogenases, α-ketoglutarate dehydrogenase, fumarase and pyruvate carboxylase or a combination of them can result in an increased KGA production and in a different product ratio of the secreted organic acids under KGA production conditions.

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[2] Holz M, Förster A, Mauersberger S, Barth G (2009) Appl Microbiol Biotechnol 81: 1087-1096
[3] Holz M, Otto C, Kretzschmar A, Yovkova V, Aurich A, Pötter M, Marx A, Barth G (2011) Appl Microbiol Biotechnol 89: 1519-1526
This study was partially co-financed by the European Union, the Land of North-Rhine Westphalia of Germany and by the Evonik Degussa GmbH.

FUP024

The secretome of *Heterobasidion irregulare* on spruce wood

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Heterobasidion species are severe pathogens in conifer plantations and natural forests in Europe and the USA and cause root and butt rot in living trees. The fungi are white rots, degrading simultaneously or selectively lignin. The genome of the North American *Heterobasidion irregular* was established by the JGI (Joint Genome Institute, Walnut Creek, CA) and the annotated genome can be used in studies of the fungal proteome. *H. irregular* was grown in liquid medium with and without *Picea abies* wood. Freely secreted and hyphal sheath associated proteins analyzed by 2D-gel electrophoresis revealed a high diversity between wood supplemented and control cultures. Protein identification by ESI-LC-MS/MS was either performed on single protein spots form 2D-gels or by application of a shot-gun method on complex protein mixtures. Using a MASCOT database with the *H. irregular* proteome as deduced from the fungal genome, in total 98 different secreted proteins have been identified. 58 proteins were present under both culture conditions and only six proteins were suppressed by wood supplementation. Addition of wood resulted in 36 new proteins secreted into the culture media. Redox-enzymes were represented by 21 proteins and most of them were induced by wood. Expression of laccases (except of one) and alcohol oxidases differed not between the two culture media. However, wood induced secretion of FAD-oxidoreductases and redox-enzymes with unknown function and furthermore induced secretion of specialized glycanases, lipases and proteases.

The JGI is gratefully thanked for providing the annotated *H. irregular* genome to the public.

FUP025

Differential gene expression in submerged spore forming cultures of the entomopathogenic fungus *Metarhizium anisopliae*

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Hyphomycetes of the genus *Metarhizium* (Ascomycota: Clavicipitaceae) are among the best characterized fungal entomopathogens infecting with widely varying host specificity over 200 different insect species. Naturally, *Metarhizium* multiplies and spreads by means of asexual conidiospores formed from mycelia on solid surfaces, e.g. an insect cadaver.
Application of *Metarhizium* and related fungi as insect biocontrol agents relies on the production of high numbers of both viable and virulent spores, and liquid as opposed to solid surface fermentation would be the preferred way to reach this goal for both technical and economic reasons. However, fungal sporulation in submerged culture must be expected to be physiologically different from solid surface sporulation. While factors involved in conidiospore formation have previously been investigated for filamentous fungi, few is known about the molecular biology and genetics of submerged spore formation.
In order to identify genes differentially transcribed in two liquid cultures of the same isolate of *Metarhizium anisopliae,* strain Ma43, - one culture reproducibly sporulating, the other showing formation of mycelial "pellets", i.e. a common form of submerged mycelial growth of this fungus - we have used a suppression subtractive hybridization PCR (SSH-PCR) approach. Methodological aspects and the current state of data mining from the project will be presented.

FUP026

Proteins expressed during hyphal aggregation for fruiting body formation in Basidiomycetes

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The first visible step in fruiting body development in basidiomycetes is the formation of small hyphal knot by localized intense branching of hyphae of restricted length followed by hyphal aggregation. In *Coprinopsis cinerea*, the first not yet fruiting-specific step of hyphal branching occurs in the dark, the second step requires a light signal. Hyphal aggregation implies cell-cell contacts and protein interactions on the outer cell walls are anticipated. Few protein candidates were identified and discussed in the past for such function, amongst were the galectins in *C. cinerea* and the Aa-Pri1 protein (aegerolysin) in *Agrocybe aegerita* that are specifically expressed during the step of hyphal aggregation as well as during subsequent primordia development. In this study we follow up the distribution of such genes in the steadily growing number of available genomes of basidiomycetes. Neither galectin genes nor *Aa-pri1*-like genes are present in all mushroom species, making an essential role in hyphal aggregation unlikely.

FUP027

Proteomic analysis of tomatoes in response to endophytic *Phialocephala fortinii* infection

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Phialocephala fortinii is a common root endophyte of woody plants and is characterized by its low host specificity. So far, little is known about the molecular mechanism of its interaction with the host and whether it is of pathogenic, parasitic, symbiotic or mutualistic nature.
In contrast to truly phytopathogenic fungi such as *Alternaria solani*, a *P. fortinii*-isolate from larch clearly promotes the growth of tomato (*Lycopersicum esculentum*) plants. A semi-quantitative metaproteomics approach was employed to investigate the molecelular mechanisms underlying the plant response to presence of the endophytic *P. fortinii* and the pathogenic *A. solani* together with the response of *P. fortinii* to plant defence mechanisms. To this end proteins were extracted from (I) *P. fortinii* and *A. solani*-infected plants, (II) uninfected control plants and (III) *P. fortinii* and *A. solani* grown on agar plates and analyzed by a combination of one-dimensional gel-electrophoresis, liquid chromatography and tandem mass spectrometry. Subsequently, 809 proteins were assigned to either plant or fungal origin and to different functional classes employing our newly established bioinformatics workflow ProPHANE (Schneider et al., 2011).
Most importantly, typical plant defence mechanisms, for instance the biosynthesis of jasmonic acid indicating induced systemic resistance (ISR), the upregulation of the pathogenesis-related protein TSI (indicating systemic acquired resistance (SAR)), the increasing amounts of beta1,3-glucanases and several chitinases, or the expression of oxidative stress

defence enzymes, were found to be induced by both fungi. Altogether, the similar responses of Lycopersicum esculentum to *P. fortinii* and *A. solani* indicatea classification of the endophyte *P. fortinii* as a pathogen by tomato plants.

Schneider, T., Vieira de Castro Junior, J., Schmid, E., Cardinale, M., Eberl, L., Grube, M., Berg, G., **Riedel K.** (2011). Structure and function of the symbiosis partners of the lung lichen (Lobaria pulmonariaL. Hoffm.) analyzed by metaproteomics. Proteomics11, 2752-2756

FUP028

Protein-protein interaction studies to decipher fungal sexual development

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Fungi are able to produce a number of different cell types and multicellular structures during their life cycle. One prominent example is the formation of fruiting bodies to propagate sexually. Our studies focused on the filamentous fungus *Sordaria macrospora* which produces fruiting bodies within seven days under laboratory conditions. By analyzing sterile mutants, several proteins required for sexual development were identified, the so-called "PRO" proteins [1]. To gain more insight into the regulation of fruiting body differentiation, we performed protein-protein interaction studies with several PRO proteins using yeast-two-hybrid, TAP-tag purification and subsequent mass spectrometry, co-immunoprecipitation, and fluorescence microscopy. We have further tested different fungal promoters for expression of recombinant proteins. Interestingly, we were able to link several developmental proteins via shared interaction partners and propose the formation of multi-protein complexes containing developmental and signaling proteins. In conclusion, our data hint to an extensive network regulating cellular differentiation in a fungal model system.

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FUP029

Optimisation of vectors for transformations in *Coprinopsis cinerea*

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The genetic transformation of the model fungus *Coprinopsis cinerea* allows the genomic analysis and manipulation of this organism. Initially, transformations were used to study the structure; functions and regulation of expression of genes; in recent years usage for overexpression of industrially important enzymes are also emerging. For the transfer of genetic material, chromosomal integrative vectors are used. These vectors contain a selectable marker gene and/or a gene of interest under the control of regulatory sequences such as promoter or terminator. Due to lack of systematic experimental data, little is known about the influence of vectors on transformation frequencies. This work targets at improvement of the transformation vector pCc1001 (1). This pUC9-based vector contains a 6.5 kb *Pst*I genomic fragment of *C. cinerea* with the tryptophan synthetase gene (*trp1*) that can be used to complement *trp1*- defects. The vector however shows a surprising phenomenon. In single transformation it gives only low numbers of transformants whereas efficiencies in co-transformation raise by factors of >100%, yielding several hundreds of transformants per experiment. To investigate this phenomenon further, the vector was modified in length and fragments with thetrp1gene were subcloned into pBluescriptKS-. The effects on the transformation efficiency were investigated by using several co-transformation experiments.

(1) Binninger DM et al. (1987)DNA-mediated transformation of the basidiomycete *Coprinus cinereus*. EMBO J 6:835-840

FUP030

A mating loci in *Coprinopsis cinerea* differ in the numbers of HD1 and HD2 homeodomain transcription factor genes

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The 25 kb-long *A* mating type locus in the mushroom *Coprinopsis cinerea* controls defined steps in the formation of a dikaryotic mycelium after mating of two compatible monokaryons, as well as the formation of the fruiting bodies on the established dikaryon. Usually, three paralogous pairs of divergently transcribed genes for two distinct types of homeodomain transcription factors (termed HD1 and HD2 after distinguished homeodomain sequences) are found in the multiple alleles of the *A* locus. For dikaryon formation and regulation of sexual development, heterodimerization of HD1 and HD2 proteins from allelic pairs from different *A* loci is required. In some *A* loci found in nature, alleles of gene pairs are not complete or one of two genes have been made in-active. Functional redundancy allows the system still to work as long as an *HD1* gene in one and an *HD2* in the other allelic gene pair are operative. In this study, we present for the first time two completely sequenced *A* loci. Evidences for gene duplications, deletions and inactivations are found. The loci differ in the number of potential gene pairs (five versus three), in genes that have been duplicated in evolution, in genes that have been lost in evolution and in genes that are still present but have been made inactive.

Kües U, James TY, Heitmann J (2011) Mating type in Basidiomycetes: Unipolar, bipolar, and tetrapolar patterns of sexuality. In: Pöggeler S, Wöstemeyer J (eds) Evolution of fungi and fungi-like organisms. The mycota XIV. Springer, Berlin, pp 97-160

FUP031

Enzymatic oxidation of nitrophenols by a DyP-type peroxidase

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The jelly fungus *Auricularia auricula-judae* produces a dye decolorizing peroxidase (DyP; EC 1.11.1.19) in plant-based complex media (e.g. tomato juice suspension). DyP-type peroxidases represent a separate superfamily of heme peroxidases and were first described for basidiomycetous fungi and later also found in eubacteria. These enzymes oxidize diverse synthetic and natural dyes including recalcitrant anthraquinone derivatives (e.g. Reactive Blue 5), as well as typical peroxidase substrates such as ABTS and 2,6-dimethoxyphenol. As lignin peroxidases (LiP; EC 1.11.1.14), some DyPs have been shown to oxidize methoxylated aromatics with high-redox potential such as veratryl alcohol and a non-phenolic β-O-4 lignin model dimer (Liers et al. 2010). To substantiate this finding, the oxidation of several mononitrophenols were tested using the DyP of *A. auricula-judae*. For peroxidases, the conversion of these high-redox potential substrates has so far only been reported for LiP. The *Auricularia* DyP was found to act on i) ortho-nitrophenol (*o*NP), ii) meta-nitrophenol (*m*NP) and iii) para-nitrophenol (*p*NP). The pH dependency for *p*NP showed an oxidation optimum at pH 4.5, which is typical for phenol conversions by DyPs. In all cases, the corresponding quinones and dinitrophenols were identified as major products of NP oxidation; moreover, the formation of further unknown products was observed in the HPLC elution profiles. The mechanism of nitration was examined using ^{15}N-labeled *p*NP and an additional source of nitro-groups (sodium nitrite). Products were identified by HPLC-MS, and mass-to-charge ratios evaluated to clarify the origin of nitro-groups. The additional nitrogen in dinitrophenols formed during enzymatic conversion was found to originate from both nitrophenol and sodium nitrite. Based on these results, a hypothetical reaction scheme has been postulated.

FUP032

Insights into gene regulation under hypoxia in the human pathogenic fungus *Aspergillus fumigatus*

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The saprophytic mold *Aspergillus fumigatus* is the predominant airborne fungal pathogen causing locally restricted pulmonary diseases such as noninvasive aspergilloma or systemic infections in immunocompromised individuals. *A. fumigatus* is usually acquired by the inhalation of fungal spores which, if not cleared by the immune system, germinate to form hyphae. At the site of infection very often inflammatory, necrotic lesions occur, which are in many cases associated with severe hypoxia. Only little is known about the regulatory circuits involved in the adaptation of this fungus to these low oxygen environments. Recently SrbA was identified as a homologue of the sterol regulatory element binding protein SREBP from fission yeast, activating hypoxic gene expression in response to low sterol levels. This mutant was unable to grow in the presence of less than 5% O_2 and attenuated in virulence in mouse infection models of invasive aspergillosis (1). Our initial experiments analyzed the gene expression of *A. fumigatus* during its long term response to hypoxia in a glucose limited O_2-controlled fermenter (2). Differential mRNA levels of a number of genes during hypoxia were verified by Northern hybridization. When analysing their expression in an srbA null mutant background we found that several of these genes were regulated independent from SrbA during a short-term exposure to hypoxia. From microarray data and genome wide searches we are aiming to identify new regulatory proteins. By characterizing the phenotypes of mutants in the respective genes their putative roles in the response to low O_2 concentrations are discussed.

(1) Willger, S.D. *et al*., 2008. *PLoS Pathog*. 4(11):e1000200.
(2) Vödisch *et al*., 2011. *J. Proteome Res*. 10(5):2508-2524.

FUP033

A proteome reference map of *Aspergillus nidulans* and new putative targets of the AnCF complex

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The mould *Aspergillus nidulans* is a well suited model organisms for filamentous fungi and is closely related to many *Aspergillus* species of industrial and medical interest. With the completion and publication of the *A. nidulans* genome it is feasible to study gene expression and protein production on a global scale. A variety of transcriptome studies have been already carried out for *A. nidulans*. By contrast, only little information is available about the dynamic changes of the proteome of *A. nidulans* upon environmental changes, stress conditions or genetic modifications. Furthermore, no proteome map for *A. nidulans* has been published so far.
For this reason, we established the first 2-D reference map for the intracellular protein fraction of *A. nidulans* strain TNO2A7. After 2D-gel electrophoretic separation, visualisation of proteins by Coomassie staining and image analysis with Delta 2D, 435 spots representing 364 different proteins were identified by MALDI-TOF-MS/MS analysis. Qunatitative proteomic analysis of a *hapC* deletion mutant revealed many proteins with difference in abundance in comparison to the wild type. Two proteins, a conserved hypothetical protein and a guanine nucleotide dissociation inhibitor, were found to be putative, so far uncharacterised targets of the AnCF complex. Knock-out and double knock-out strains of the corresponding genes are in progress

FUP034

Interaction of the phytopathogenic fungus *Verticillium longisporum* with the antagonistic soil bacterium *Pseudomonas fluorescens*

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The filamentous soil-borne fungus *V. longisporum* possesses phytopathogenic properties and is responsible for increasing economical losses in the cultivation of oilseed rape (*Brassica napus*). Antagonistic bacteria like *Pseudomonas fluorescens* can be used as biological control agents to reduce the infection intensity of saprophytic fungi in the absence of appropriate fungicides. We analyse the interaction between these three organisms on a molecular and genetic level to explore the potential of *P. fluorescens* as a biocontrol tool for *V. longisporum*. Initial experiments suggested that the impact of the bacterium on fungal growth is highly medium and strain-dependent. We focused on potential bacterial mycotoxins. Biosurfactants (glucolipids or cyclic lipopeptids) and phenazines produced by *P. fluorescens* are acting against resting structures of fungal pathogens. They are operating synergeticly by integrating the biosurfactants into the cell membrane increasing its permeability for the toxic phenazines to enter into the cytoplasm. The bacteria produce a variety of additional antifungal secondary metabolites like 2,4-diacetylphloroglucinol, hydrogen cyanide or pyrrolnitrin which are all controlled by the two-component system *gacS-gacA*. *P. fluorescens* mutants defective in the production of single secondary metabolites tested were still able to inhibit the germination of fungal spores. Knockouts of the general regulator *gacA* of *P. fluorescens* lost the ability to inhibit germination of the fungus. Our future focus will be the fungal response to the presence of the bacterium.

HMV001

Deglycosylation of polyphenolic *C*-glucosides by a human gut bacterium

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Dietary polyphenols, such as (iso)flavonoids, have been implicated in the prevention of age-related chronic disorders including cancer and cardiovascular diseases [1]. Polyphenols are present in plant-derived food and food supplements, predominantly in their glycosidic form, either as *O*-glycosides or as *C*-glycosides. In contrast to the *O*-glycosides, ingested *C*-coupled glycosides resist cleavage by human enzymes and can most likely only be deglycosylated by gut bacteria. A rod-shaped Gram-positive bacterium, strain CG19-1, capable of deglycosylating the isoflavone puerarin (daidzein 8-*C*-glucoside) to daidzein was isolated from human feces [2]. Comparative 16S rRNA gene sequence analysis indicated that the strictly anaerobic isolate is a new species of the *Lachnospiraceae*. Strain CG19-1 also converted polyphenolic *C*-glucosides other than puerarin. The xanthone *C*-glucoside mangiferin was deglycosylated to norathyriol. Several *C*-glucosides of the flavones luteolin and apigenin were cleaved to their aglycones, which were further degraded to the corresponding hydroxyphenyl propionic acids. Strain CG19-1 also converted (iso)flavonoid *O*-glucosides, but at rates that were lower than those observed for the *C*-glucosides. The isoflavone *O*-glucosides were converted to their aglycones, while the flavone *O*-glucosides underwent deglycosylation and subsequent degradation to hydroxyphenyl propionic acids. Thus, strain CG19-1 may affect the bioavailability and, thereby, the effects not only of polyphenolic *O*-glucosides but also of *C*-glucosides previously assumed to be stable in the human body. The mechanism of the *C*-glucosyl cleavage remains to be elucidated. For this purpose, identification of the involved enzyme(s) from strain CG19-1 is in progress.
[1] Crozier A, Jaganath IB, Clifford MN (2009) Nat Prod Rep 26: 1001-43
[2] Braune A, Blaut M (2011) Environ Microbiol 13: 482-91

HMV002

Application of real-time PCR, T-RFLP and direct sequencing for the identification of polybacterial 16S rRNA genes in ascites

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Question: Spontaneous bacterial peritonitis (SBP) is a serious complication in cirrhotic patients with a mortality rate up to 50%. However, early diagnosis and antibiotic treatment can improve clinical outcome. Due to the limited detection rates of culture-dependent bacterial identification in patients with clinical SBP diagnosis, we evaluated 16S rRNA gene amplification for the rapid detection of bacterial DNA in ascites and further characterized polybacterial samples by terminal restriction fragment length polymorphism (T-RFLP) and direct sequencing.
Methods: 98 ascitic fluid samples from 43 patients undergoing several diagnostic paracenteses were studied. To avoid cross hybridization of bacterial broad range primers with the human DNA background we selectively isolated bacterial DNA of all samples with a commercially available isolation kit (MolYsis). 16S rRNA genes were amplified by real-time polymerase chain reaction (PCR) and directly sequenced. Using the web-based tool RipSeq (iSentio), mixed chromatograms were immediately interpreted. T-RFLP analysis characterized polymicrobial samples by displaying their bacterial diversity patterns.
Results: Bacterial DNA (bactDNA) was detected in 57/98 (58%) of the ascitic fluid samples. 22/43 patients (51%) underwent several paracenteses (mean 3.5; range 2-6) from which 5/22 patients (23%) showed positive bactDNA in ascites throughout all paracenteses and 4/22 (18%) patients were bactDNA negative. In the remaining 13 patients at least one positive ascites sample could be detected (mean number of positive samples 2.3; range 1-4). A single paracentesis was performed in 21/43 (49%) patients. BactDNA positive ascites was observed in 12/21 (57%) samples whereas 9/21 (43%) ascitic fluids were PCR negative. Using TRFLP, multiple T-RF were detected in positive ascites potentially indicating the presence of several distinct strains. Direct sequencing with 16S rRNA gene based primers showed mixed chromatograms which revealed gram positive as well as gram negative organisms.
Conclusion: A mixed bacterialDNA content can bedetected in ascites via PCR targeting the 16S rRNA genes and T-RFLP analysis. Direct sequencing of PCR products and analysis of mixed chromatograms using RipSeq may offer a rapid tool to identify the most abundant sequence types.

HMV003

Analysis of the intestinal microbiota using SOLiD 16SrRNA gene sequencing and SOLiD shotgun sequencing

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Metagenomics seeks to understand microbial communities and assemblages by DNA sequencing. Technological advances in next generation sequencing technologies are fuelling a rapid growth in the number and scope of projects aiming to analyze complex microbial environments such as marine, soil or the gut. Recent improvements in longer read lengths and paired-sequencing allow better resolution in profiling microbial communities. While both 454 sequencing and Illumina sequencing have been used in numerous metagenomic studies, SOLiD sequencing is not commonly used in this area, as it is believed to more suitable in the context of reference-guided projects. To investigate the performance of SOLiD sequencing in a metagenomic context, we compared taxonomic profiles of both Sanger and SOLiD mate-pair sequencing reads obtained from the bacterial 16S rRNA gene that was amplified from microbial DNA extracted from a human fecal sample. Additionally, from the same fecal sample, complete genomic microbial

DNA was extracted and shotgun sequenced using the SOLiD technique to study the composition of the intestinal microbiota and the existing microbial metabolism. By analyzing the data using BLASTX against the NR database and MEGAN we found that the microbiota composition of 16S rRNA gene sequences obtained using Sanger sequencing and SOLiD sequencing provide comparable results. However, with SOLiD sequences we obtained more resolution down to the species level. In addition, with the shotgun data we are able to identify the functional profile using SEED and KEGG.
This study shows that SOLiD mate-pair sequencing is a viable and cost efficient option for analyzing a complex microbiome. To the best of our knowledge, this is the first time that SOLiD sequencing has been used in such a study.

HMV004

Bacteriocin production of staphylococcal nasal isolates

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Staphylococcus aureus is a major pathogen in hospital- and community-acquired infections. Colonisation of the anterior nares in about 30% of the population is a major risk factor for *S. aureus* infections. Recently the composition of the nasal flora has been inverstigated. Interestingly, the bacterial diversity in the human nose reaches from aerobic to strictly anaerobic bacteria. The most frequently occurring species are *Corynebacterium accolens/ C. macginleyi, S. epidermidis and Propionibacterium acnes*. In order to investigate if bacteriocin production might play a role during nasal colonisation, we analysed the bacteriocin production of nasal *Staphylococcus* strains.
The test-strains were casted in an agar plate and the nasal isolates were stamped on the plate. Various isolates showed growth inhibition zones of the test-strains. Transposon plasmids could be transformed into various strains and mutagenesis was performed.
Analysis of 93 stapylococcal nasal isolates offered that various strains produce bacteriocins against *Micrococcus luteus* and other nasal bacteria (*S. aureus, Corynebacteria, Moraxella, Propionibacteria*…).The bacteriocin production of some nasal isolates turned out to be inducible by hydrogen peroxide or iron limitation.
One of these bacteriocins, produced by an *S. epidermidis* strain, could be characterized as a Nukacin-like lantibiotic with activity against *Micrococcos luteus*, *Moraxella catarrhalis*, *Streptococcus pyogenes* and *Corynebacterium pseudodiphtheriticum*.
Knowledge about the various interactions between staphylococcal and other nasal isolates could be important for effective *S. aureus* control strategies.

HMP001

Microbiological air quality in the hospital environments of two major hospitals in Benin City metropolis, Edo State, Nigeria

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We spend most of our lives in different indoor environments, in homes, day-care facilities, schools and workplaces and we are constantly being challenged by the microbial contents of these environments. It became imperative to undertake a study of the microbiological air quality of the airborne microflora in the environments of two major government owned hospitals (University of Benin Teaching Hospital, (UBTH) and Central Hospital) in Benin City metropolis. The air samples were sampled every month for the three (3) months in the wet season (June - August, 2010) and three (3) months of the dry season (November 2010 - January 2011) using the settled plate methods. The study sites were divided into nine units which includes Accident and Emergency Ward, Laboratory, Male ward, Female Ward, Children Ward, Labour Room, Treatment Room, Theatre and outside the hospital gate. The mean airborne bacterial load in the two hospitals ranges from 8.5cfu/min to 172.5cfu/min and 5.5cfu/min to 64.5cfu/min for UBTH and Central hospital in the wet season. While the mean airborne fungal load in UBTH and Central hospital in dry season ranges from 2.5cfu/min to 9.5cfu/min and 1.5cfu/min to 19.0cfu/min respectively. The female ward, children ward, accident and emergency ward and outside the hospital gate were recognized to record the highest airborne microflora. The result revealed the isolation of ten (10) fungal airborne isolates and six (6) airborne bacterial isolates. These includes, Aspergillus niger, Aspergillus flavus, Botryodiplodia acerina, Rhizopus stolonifer, Nigospora zimm, Mucor sp., Monilla infuscans, Penicillium sp., Candida sp. and Trichoderma viridis, while the six (6) bacterial isolates includes Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Bacillus sp., Serratia marcescens and Micrococcus sp. The result shows the highest fungal population of 26.5cfu/min (Outside environment) in UBTH followed by 24.0cfu/min (Outside environment) in Central Hospital. The highest bacterial population of 172.5cfu/min (outside environment) was recorded in UBTH. The fungal isolates Aspergillus niger (53.0%) and Monilla infuscans (43.9%) were showed to be the most frequently isolated airborne fungal isolates while Staphylococcus aureus (91.3%) and Staphylococcus epidermidis (85.8%) were the most frequently isolated airborne bacterial isolates. The Statistical analysis showed no significant difference between the values obtained during the wet and dry seasons in both hospitals studied. KEYWORD: Airborne microflora, bacteria, fungi, hospital environment, time and bioaerosols.

HMP002

Unusual Multi organ presentation of Hydatid cyst

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Introduction: Hydatidosis is a common problem all over the world. Hydatid cysts could be formed in all parts of human body except hair and nail that there is no blood. The prevalence of this disease is higher in children rather than adults. Risk of infection depends on sanitation and it could be prevented easily. The ultimate treatment is surgery but recurrent rate can be decreased by administering medical treatment in the preoperative and post operative periods.
Case Report: A 35 year's old female patient presented with cough, purulent production and dyspnea. In computerized tomographic scan (CT scan) numerous cysts were observed in chest, abdomen and paravertebral muscles. Because of cysts were ruptured, surgical intervention was planned for thoracic lesions without prior antiparasitic medical treatment. The patient had no complications in short- term follow up.
Discussion: Hydatid cyst is a parasitic disease which is known from the time of Hippocrates. Infection will occur by eating vegetables contaminated with eggs of this parasite or contaminated viscera of herbivores and parasite larvae form cyst in human body. The infected person is in risk of pneumocysis, pleural effusion, pneumothorax, secondary Echinococcus in pleural and peritoneal cavities and muscles involvement. In our case there was platysma Dorsey and abdominal muscle involvement that there was no problem despite of dorsal and pleural cysts rupture, fortunately. Another interesting point in this case was that, there was no relation between pleural and dorsal muscle cysts. Generally observance of basic principles of health such as washing hands with soap after gardening or contact with dogs and also washing vegetables that could be contaminated with dog feces are very important points preventing these diseases. However this disease could be cured easily by surgery, if surgery is conducted after taking a short course of Albendazole and Mebendazole, the efficacy of surgical treatment will be better.

HMP003

Relationship among chlamydia pneumoniae infection, atherosclerosis and expectancy of coronary artery disease

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Introduction: Coronary artery disease (CAD) is the leading cause of death in many countries. The underlying mechanism of the chronic inflammatory process in atherosclerosis is still unknown (1). But, the risk for coronary events may rise during acute infection (2). There are findings suggesting the inflammatory and immunogenic nature of the atherosclerosis (3). It is currently unclear what causes the chronic inflammation within atherosclerotic plaques. One emerging paradigm suggests that infection with bacteria and/or viruses can contribute to the pathogenesis of atherosclerosis (4). Chronic Chlamydia pneumoniea infection has recently been associated with atherosclerosis. The aim of this study was to investigate the association of Chlamydia pneumoniea infection, Ischemic heart disease (IHD) and atherosclerosis.
Material and Method: 86 patients with background of recent Ischemic Heart Disease, referred to Shahid Madani hospital of Tabriz from January 2010- January 2011 were studied. Different blood samples were taken to assess the lipid profile and other tests. Blood culture was done and Triglyceride, High-Density Lipoprotein (HDL), Low density Lipoprotein (LDL), IgG and IgA antibodies against Chlamydia Pneumoniea were

measured by conventional enzymatic methods and micro immune fluorescence method. SPSS version 17 software was used for analyzing the data.
Results: Antibody test against Chlamydia pneumoniea was positive in 41 patients (47.67%) and were diagnosed as seropositive. Confirming no significant difference between seropositive and seronegative patients in HDL (p=0.4), mean concentrations of Total cholesterol, LDL and Triglyceride were significantly higher in Chlamydia pneumonia seropositive patients respectively. The mean concentrations of triglyceride, Total Cholesterol and LDL were P=0.003, P=0.001 and P=0.005. To see the relationship among IHD, infection and lipid profile multivariate analysis were done that, it was also following the univariate results.
Conclusion: Thus, C. pneumoniae antibodies seem to correlate with an altered serum lipid profile considered to increase the risk of atherosclerosis. This finding supports the proposal that C.pneumoniae infection may play a role in the pathogenesis of atherosclerosis (5). In some cases, the infectious agents are found within the plaques and viable organisms can be isolated suggesting a direct effect (4). Also, C. pneumoniae antibody positivity was independently associated with ischemic stroke in elderly patients (6). Chronic Chlamydia pneumoniea infection was one of the risk factors for Ischemic heart disease, but to know its exact role and mechanism, more studies are required.

HMP004

Role of *Pseudomonas aeruginosa* in nasocomial infections and approach to its treatment

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Introduction: One of the most important concerns in hospitals is antimicrobial resistance in hospital pathogens that puts patients in risk of morbidity and mortality. It is caused by plasmid mediated resistance against beta lactams by producing extended spectrum beta lactamases enzyme (ESBLs). Pseudomonas aeruginosa is one of those pathogens that is in common with most of these nasocomial infections. This study was conducted to find the role of Pseudomonas aeruginosa in nasocomial infections to find the best approach for its treatment.
Methods: Different Samples from different parts of like tracheal aspirate, urine, blood, bronchial aspirate, sputum, CSF, wound discharge, bone marrow and peritoneal fluid of ICU patients of 5 hospitals in Tabriz were taken. They were tested for susceptibility by Disk agar diffusion method and screening of ESBL-producing by Double disc approximation test, respectively, also combined test disc method and MIC determination by E-test were adopted for confirmation. Extracting Plasmid DNA by Kado and Liu technique, the presences of bla $_{CTX-M1}$, bla $_{CTX-M2}$ were studied by Polymerase Chain Reaction (PCR).
Results:248 ICU patients, infected by Gram-negative bacilli were studied. Pseudomonas aeruginosa was the second agents in nosocomial infection, 67 (27%). The susceptibility test showed 29%, 38%, 53.9%, 98%, 96%, 70% 100% and 100% resistance against Piperacillin, ceftazidim, Ofloxacin, Sulphamethoazle, Cefotaxime, ceftriaxone, Tetracycline and cefuroxime. The Double Disk Test showed 96.7%, 100% and 96.6% resistance against Ceftriaxone, Cefotaxime, and Ceftazidime. The combined Test showed 63.4% negative result against Cefotaxime and Cefotaxime / Clavulanic acid and 67.5% against Ceftazidime and Ceftazidime / Clavulanic acid. By E-test ESBLs production was detected in P.aeruginosa(78%). In plasmid extraction 64.5 % of isolates harbored a single plasmid of 63kb.On the basis of PCR results, All of strains lacked either CTX-M-1 or CTX-M-2 gene to confirm the rule for bla$_{CTX-M}$.
Conclusion:Pesudomonas aeruginsawas one of the most prevalent bacteria. Highest rate of resistance was showed against Cefuroxime, Tetracycline and lowest rate was showed against Amikacin and Piperacillin. Our results showed that DDT test was not as sensitive as CT and MIC methods and no statistical significant difference was found between results of CT and MIC. Confirming no rules for suspicious genes by PCR, 78% of strains were founded as ESBL producer. Since the genes encoding theses enzymes are mainly located on plasmids, so transmission of the plasmids could disseminate the resistance in future, unless the consumption of cephalosporins are restricted and antibiotics such as imipenem substituded for the third generation cephalosporins, because these antibiotics, especially ceftazidim and ceftriaxone are strong inducers of ESBLs.

HMP005

Identification of D-tryptophan as immunologically active compound excreted by probiotic bacteria using immunological in vitro-test systems

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The interest of probiotic bacteria in health care is increasing in the last years. However there is still a lack of understanding the underlying mechanisms. One reason for the detected health benefits might be the crosstalk between bacteria and the host which is difficult to predict. Therefore, we aimed to identify soluble compounds produced by probiotic bacteria using in vitro screening tools, high performance chemical analysis and metabolic profiling.
Gram-positive probiotic bacteria were grown in defined minimal medium and supernatants were taken at stationary phase. Culture supernatants, separated fractions and pure compounds were screened for their ability to prevent the lipopolysaccharide-induced maturation of human monocyte-derived dendritic cells (DC). The activation markers CD83, CD86, CD80, and CD40 were measured with flow cytometry. Furthermore tests were performed indicating inhibition of the allergy-related thymus and activation regulated chemokine (TARC) secretion of KM-H2 Hodgkin Lymphoma cells (ELISA). Immune-active supernatants were fractionated by solid phase extraction and elution was performed with increasing methanol concentration in water. After separation immune-active fractions and compounds were analysed using FTICR-MS and NMR.
Supernatants of several bacterial strains significantly down-regulated the expressing of the activation markers CD83, CD86 and CD40. They also significantly reduced TARC secretion of KM-H2 cells. After fractionation of two selected supernatants, the immune modulatory activity was found in the 20%, 40% and 50% methanol fractions. We focused on the compound in the 20% methanol fraction and were able to characterize it as D-tryptophan. In addition it was the only D-amino acid having this immune modulatory effect when we tested the pure D-amino acids.
The present work demonstrated that small extracellular molecules from probiotic strains have immune modulatory activity in the screening systems applied. Our work provides evidence that D-tryptophan is one of several hitherto not yet described small molecules of probiotic bacteria which potentially interfere with human immune responses. This may give further basis for the application of these compounds as food additives to finally provide anti-allergic effects.

HMP006

Metabolic characterization of dental biofilms produced by *Streptococcus mutans* under dietary carbohydrate exposure

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Strep. mutans is known to be a major pathogen for dental caries and human tooth decay. Especially, sucrose induces high cariogenicity by activating microbial glycosyltransferases and thus production of extracellular adhesive polysaccharides. The aim of the study was the metabolic characterization of *Strep. mutans* biofilm formation in the presence of glucose, sucrose and the sugar alcohol xylitol. Biofilms of *Strep. mutans* were produced metabolizing Schaedler-broth (0.58% glucose) (M1), Schaedler-broth with 5% sucrose as supplement (M2) and Schaedler-broth wih 1% xylitol in addition (M3) on human enamel slides. After 24h incubation time at 37°C the surface-associated biofilms were labeled using three specific staining protocols: 1. live/dead cell differentiation, 2. staining of total bacterial cells and extracellular polysaccharides (EPS) by concanavalin A and 3. staining of total bacterial cells and microbial respiratory activity. The marked biofilms were analysed by confocal laser scanning microscopy and checked for microbial total cell counts and colony growth on Schaedler agar plates. Ten series of each approach were performed and analysed by means of one-way analysis of variance and Tukey Kramer statistical tests. The streptococcal biofilm thickness and volume reached its maximum under sucrose exposition in M2. Regarding the stratification of the biofilms the ConA-based EPS-signals in all three media (M1-M3) showed higher acitivity in the internal regions of biofilms near to enamel compared with outer biofilm regions. The microbial respiratory activity tended to be lower in M2 in comparison with M1 and M3. In the presence of sucrose *Strep. mutans* biofilms appeared as microcolonies associated with increased viability parameters like biofilm depth, volume and higher vitality proportions compared to the corresponding biofilms grown in media with glucose or xylitol. Within the

laboratory conditions the non-cariogenic sweetener xylitol did not reveal decrease of microbial vitality, respiratory activity or EPS-activity.

HMP007

Flagellin and tcpC are essential factors of the protective effect of *E. coli* Nissle strain 1917 in DSS- induced colitis

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Background: The probiotic *E. coli* Nissle strain 1917 (EcN) is as effective as mesalazine in maintenance of remission in ulcerative colitis and shortens the duration of diarrhea in young children. We studied in a preclinical model of acute colitis whether EcN protects from disease and analysed the bacterial mechanism underlying the anti-inflammatory capacity.
Methods: C57BL/6 and TLR5$^{-/-}$ mice were fed with either EcN or EcNΔfliC or EcNΔtcpC and treated with 3, 5% DSS. Body weight and disease activity index were assessed daily. At the end of the experiment the colon length and weight was measured and inflammation was determined by histological analyses of the colon. Furthermore activation and maturation of lamina propria and mesenteric lymph node dendritic cells and T cells was analysed.
Results: In wild type mice *E. coli* Nissle protects from DSS induced colitis whereas the protection is reduced in TLR5$^{-/-}$mice. In line with this the ΔfliC mutant strain was less effective in protecting the host from disease as compared to the EcN wild type strain. However a second bacterial factor tcpC also contributes to the protective effect of EcN as the ΔtcpC mutant strain was not able to protect from disease. Administration of the double mutant ΔfliCΔtcpC of EcN evidences this conclusion.
Conclusions: EcN ameliorates a DSS induced acute colitis via flagellin and the secreted protein tcpC. However contribution of further bacterial factors to the anti-inflammatory effect of EcN can not be excluded.

HMP008

Molecular mechanisms leading to semi-mature murine dendritic cells and their role in intestinal homeostasis

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Dendritic cells (DCs) can provide different phenotypes. They can display an immature DC (iDC) phenotype or an activated mature DC (mDC) phenotype. Recently a third phenotype has been discovered, termed semi-mature (smDCs). These smDCs are able to take up antigen, but not to process it and they show reduced expression of T cell activating co-stimulatory molecules and a reduced expression of MHC class II. SmDC fail to polarize T cells. Sm BMDCs show reduced cleaving of the invariant chain (Ii) compared to mDCs, a major regulator of the MHC class II transport to the cell surface, so leading to reduced MHC class II surface expression. The cleaving of Ii is catalyzed by the endosomal protease CatS, which is regulated by the endogenous inhibitor Cystatin C. Indeed, mice lacking Cystatin C provide a significant higher susceptibility towards DSS induced colitis.
Therefore we suggest, that semi-maturation plays an important role in maintaining the intestinal homeostasis and that regulation of Cathepsin S could be a potential target for the treatment of colitis.

HMP009

Role of dendritic cell activation as well as Toll-like receptor 2 and 4 expression while DSS colitis

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Hosts live normally in symbiosis with their intestinal microbiota due to a long co evolution. Somehow this tolerance is abolished in IBD patients, which leads to a strong and long lasting inflammation irregularly interrupted by short remission phases.
In order to investigate the role of dendritic cells and their TLR2 and TLR4 expression while acute phase inflammation the Dextran sodium sulfate (DSS) model was employed. Therefore C57BL/6 mice were treated with 2.5 % (v/w) DSS for 6 days. DSS treated mice featured and increased surface expression of TLR2 and TLR4 on lamina propria dendritic cells (LPDC) compared to healthy mock mice. The role of TLR4 signaling was elucidated precisely by additional administration of *E. coli* JM83 or *E. coli* JM83 Δ*htrB htrB*$_{Pg}$ to mice during DSS challenge. The lipid A structure of *E. coli* JM83 Δ*htrB htrB*$_{Pg}$ features a palmitate instead of laurate and is therefore less endotoxic and induces a weaker TLR4 signaling. Mice treated with DSS and *E. coli* JM83 administration showed and reduced weight loss, disease activity index and reduced colon shortening compared to DSS treated and *E. coli* JM83 ΔhtrB htrB$_{Pg}$ administered or DSS only treated mice. DSS treatment and administration of *E. coli* JM83 led as well to an increased gen expression level of anti-inflammatory genes compared DSS treatment and *E. coli* JM83 Δ*htrB htrB*$_{Pg}$ administration. The activation level of LPDC was not important concerning disease severity, since all DSS treated mice independent of bacterial administration featured high surface expression of MHCII, CD40, CD80 and CD86. Of further interest was the distribution of LPDC into subsets, therefore cells were analyzed for their surface expression of CD8, CD4, CD11b and CD103. The only difference in subset distribution was the increased percentage of CD103^{+} DC in the LP as well as in the mesenteric lymph nodes of mice receiving DSS and *E. coli* JM83 compared to mice receiving DSS and *E. coli* JM83 Δ*htrB htrB*$_{Pg}$. DSS treated and *E. coli* JM83 administered mice showed similar surface expression of TLR2 and reduced surface expression of TLR4 compared to DSS treated and *E. coli* JM83 Δ*htrB htrB*$_{Pg}$ administered mice
Increased appearance of CD103+ DCs and higher amounts of TLR2 and TLR4 are likely to be a counter regulation of the host in order to suppress developing inflammation.

HMP010

Clonal diversity and geographic signatures of human oral bacterial strains on a world-wide scale

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Objective: The human microbiome projects seek to describe the bacterial communities harboured in the human body. From a medical perspective this research has revealed the importance of human-associated microbial communities for health or disease. However, viewing the human microbiome from an evolutionary perspective can provide valuable information regarding the history of our ancestors (i.e. human migration pattern). The most prominent and as yet successful example is Helicobacter pylori as its genetic variants could well be correlated with distinct human populations. The basic drawback of H. pylori related studies however is the requirement of stomach-biopsies which drastically reduces the number of samples that can be analyzed. Here we test the hypothesis that the genetic variability of distinct bacterial species in the oral ecosystem may have the similar potential as a chronometer of human evolution. Methods: To this end saliva samples from ten volunteers each from 12 areas world-wide, representing diverse ethnic groups have been the initial focus of this study. Variations in the 16S-23S rDNA internal transcribed spacer region of Fusobacterium nucleatum and the gdh, (encoding for the glucose-dehydrogenase) and the gtf (encoding for the glucosyl-transferase) of the mitis-streptococci (all of which are typical pioneers of biofilm formation) have been analyzed by culture-independent methods. Results: As a result we observed a high intra- and inter-individual clonal diversity for all species analyzed. Phylogenetic tree reconstruction revealed several clusters shared between two or more countries but also country-specific lineages. Using the Unifrac significance test and the P-test showed significant differences between strain populations and geographic regions. The degree of those differences however varied among the genes analyzed and the countries included. Conclusions: So far it remains unclear to what extent the differences are due to divergence and vertical inheritance or rather due to diet and/or geography influences. However the data indicate that the salivary microbiome may hold valuable information for providing new perspectives on unsolved human migration patterns - an issue of medical, social and anthropological importance.

HMP011

Effects of antimicrobial peptides on methanogenic archaea

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Methanogenic archaea occur as members of the indigenous human microbiota found on several mucosal tissues. Therefore they are exposed to antimicrobial peptides (AMPs) secreted by these epithelia. Although the antimicrobial and molecular effects of AMPs on bacteria are well described, data for archaea are in general not available yet. As the archaeal cell envelope differs profoundly in terms of chemical composition and structure from that of bacteria it is not evident whether AMPs affect them.
The effects of different natural and synthetic AMPs on the growth of *Methanobrevibacter smithii*, *Methanosphaera stadtmanae* and *Methanosarcina mazei* strain Gö1 were tested with a microtiter plate assay that had to be adapted to their anaerobic growth requirements and allows measuring growth curves. Overall the tested methanogenic archaea were highly sensitive against the used cathelicidins, lysins and one synthetic peptide, however the sensitivities to the AMPs differed markedly among the different strains. Atomic force microscopy and transmission electron microscopy revealed that the structural integrity of the archaeal cells is destroyed within 4 hours of incubation with AMPs. Using the LIVE/DEAD stain the disruption of the cell envelope of *M. smithii*, *M. stadtmanae* and *M. mazei* within a few minutes could be verified. Our results strongly suggest that the release of AMPs by eukaryotic cells is a potent defence mechanism not only against bacteria, but also against methanogenic archaea.

HMP012

Characterization of naturally occurring, industrial and medical relevant biofilms

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Both abiotic and biotic surfaces are subject to bacterial colonization and biofilm formation. Biofilms formed on engineered surfaces or in medical context can cause material degradation, fouling or infections. To provide insights into various microbial biofilms, naturally occurring biofilms such as microbial consortia on the widely distributed moon jellyfish *Aurelia aurita*, a glacial biofilm, industrial and medical relevant biofilms were characterized. Biofilm compositions were studied by 16S rDNA phylogenetic analysis revealing only a very limited number of bacterial species in case of the *A. aurita* consortia indicating specific interactions (attraction/defence) between the host and the microorganisms. The main part of the analyzed sequences from the glacial biofilm yielded homologies to uncultured bacteria found in contaminated habitats that are potentially involved in bioremediation processes. Analysis of supragingival biofilms from different persons showed in general high microbial diversity, however they differed in the frequency of paradontopathogenic bacteria. The frequencies of these pathogenic bacteria showed a strong correlation to the respective inflammatory reaction defined for the test persons. The obtained results may allow understanding ecological systems, e.g. host-microbe interactions, and provide insights into the prevention of detrimental biofilms in the medical sectors and industry.

HMP013

Impact of the intestinal microbiota on mucosal homeostasis

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In addition to genetic predisposition, environmental factors such as commensal bacteria contribute to the development of inflammatory bowl disease (IBD).
The gut of mammalians is colonised by a complex flora of microorganisms containing 500-1000 different bacterial species. These bacterial populations contribute to the health of the host, among other things, by promoting proper immune systeme development and limiting pathogen colonization. *Bacteroides vulgatus* mpk was shown to have the ability to prevent colitis, whereas *E. coli* mpk induces intestinal inflammation in interleukin-2-deficient (IL-2$^{-/-}$) mice. The mechanism however remains unclear.
In the current study we analyse the composition of the intestinal microbiota of T-cell transferred Rag1$^{-/-}$ mice by 454-Seqeuncing of 16S rRNA encoding genes. With this method we want to reveal differences between the gut microbiota of mice that develop colitis compared to mice that stay healthy and the composition of the intestinal microbiota before and during development of colitis.

MEV001

Mass spectrometric analysis of antibiotics from bacteria

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Thiazolyl peptids are naturally occurring antibiotics produced by several actinobacteria. The sulfur-containing, highly modified, macrocyclic peptides are some of the most potent *in vitro* growth inhibitors of Gram-positive bacteria by inhibition of protein synthesis. Because of continuously developing antibiotic-resistance of many bacteria there is still a medical need to find new antibiotics. The natural function of antibiotics is still not sufficiently clarified, but antagonistic features are assumed which occur due to interaction with other organisms. The previous antibiotic screenings were often performed under laboratory conditions and did not simulate environmental circumstances for antibiotic production, e.g. co-cultivation with other species. A reinvestigation with these modified conditions consumes time and money. To allow a fast, sensitive, and cost-effective screening of cultivable bacteria we are currently establishing a high throughput infusion mass spectrometry method in which liquid extraction surface analysis using Triversa Nanomate technology is combined with the high mass accuracy and resolution available on LTQ-OrbitrapXL tandem mass spectrometer. The screening method was evaluated using different thiazolyl peptide producing *Streptomyces* strains. The obtained data indicate that in addition to antibiotic discovery this technique can be a powerful tool for many other microbiological approaches, e.g. surface studies of signal molecules directly between different bacterial species or other microorganisms.

MEV002

Mining for new lantibiotic producer in microbial genome sequences

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The discovery of antibiotics was one of the most important milestones in medicine and in the fight against infectious disease. Today, more than 80% of anti-infective drugs are natural or semi-synthetic compounds. Rapidly developing superbugs, i.e. pathogens that are resistant to almost all commonly used antibiotics, have become an enormous problem. This necessitates a further search for new antibiotic substances and sources. To this end, bacteria and their huge potential to produce antimicrobials represent an inexhaustible resource.
Lantibiotics (lanthionine containing antibiotics) are ribosomally produced bacterial peptide antibiotics that show interesting activities even in the nanomolar range against (multiresistant) human pathogens. The characteristic thioether aa (methyl-)lanthionine is introduced by extensive enzyme-mediated posttranslational modifications. These rare aa form intramolecular rings that are essential for the three-dimensional structure of lantibiotics, their enhanced stability against proteases and oxidation, as well as antimicrobial activity. These features make lantibiotics interesting candidates or lead structures for novel antimicrobial applications in medical and food industry.
Blast searches employing characteristic lantibiotic biosynthesis enzymes (LanM,B,C) in the NCBI database showed that ORFs coding for proteins involved in lantibiotic production are widespread in bacteria of different phyla. Based on these genomic data, we identified putative lantibiotic gene clusters in bacterial strains, for some of which production of lantibiotics had never been described before. The focus of our project is the homologous and/or heterologous expression of those, so far uncharacterized, lantibiotics. In this context, we were able to identify and characterize the novel two-peptide lantibiotic lichenicidin that is produced by *Bacillus licheniformis* DSM 13. Additionally, a partial lantibiotic gene cluster coding for proteins involved in producer self-protection against the well-known lantibiotic mersacidin is present in *Bacillus amyloliquefaciens* FZB42. Transfer of the biosynthetic part of the mersacidin gene cluster to *B. amyloliquefaciens* FZB42 resulted in successful expression of fully modified and active mersacidin in this strain. Other putative lantibiotic producers, including a Caldicellulosiruptor bescii strain, were identified and are still in the focus of the ongoing work in this project.

MEV003

Biosynthesis of class III lantibiotics - in vitro studies

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Lantibiotics represent an important class of peptide natural products synthesized by large variety of Gramm positive bacteria. The most characteristic structural feature of all lantibiotics is the presence of lanthionine (Lan) bridges, a posttranslational modification, providing structural constraints necessary for the biological activity[1]. The ribosomal origin and interesting biological properties turns lantibiotics into promising templates for the design of new biologically active compounds. Recently we reported on novel class III lantibiotics named labyrinthopeptines from Actinomycetes[2]. The characteristic feature of labyrinthopeptines is a unique carbacyclic side chain linkage composed of the posttranslationally modified triamino triacid named labionin (Lab) introduced by the LabKC enzyme[3]. In addition labyrinthopeptin A2 displays a rare activity against neuropathic pain in mammals. In order to exploit unique features of the labionin biosynthesis, the activity of the modifying enzyme LabKC was reconstituted *in vitro*, allowing a detailed mechanistic investigation. The LabKC enzyme, as all class III synthetases display a unique, well defined domain arrangement in which each catalytic activity necessary for the biosynthesis can be assigned to a specific domain (see figure). It was possible to identify a recognition motif within the leader peptide, necessary for the processing by the LabKC[4]. In addition the mode of processing and the substrate specificity were investigated providing deep insights into the activity of class III enzymes. It was also found that the GTP preference of LabKC is not conserved within class III lantibiotics. We believe that large structural diversity of this class of lantibiotics and the wide spread of homologues enzymes in known genomes might result in discovering of new promising structures in the nearest future.

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MEV004

The Effect of MbtH-like Proteins on the Adenylation of Tyrosine in the Biosynthesis of Aminocoumarin Antibiotics and Vancomycin

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MbtH-like proteins, comprised of approximately 70 amino acids, are encoded in the biosynthetic gene clusters of non-ribosomally formed peptides and other secondary metabolites derived from amino acids. Recently, several MbtH-like proteins have been shown to be required for the adenylation of amino acid in non-ribosomal peptide synthesis. We now investigated the role of MbtH-like proteins in the biosynthesis of the aminocoumarin antibiotics novobiocin, clorobiocin and simocyclinone D8 as well as the glycopeptide antibiotic vancomycin. It could be shown that the tyrosine-activating enzymes CloH, SimH and Pcza361.18, involved in the biosynthesis of clorobiocin, simocyclinone D8 and vancomycin, respectively, require the presence of MbtH-like proteins in a molar ratio of 1:1. They form a heterotetramer consisting of two adenylating enzymes and two MbtH-like proteins. In contrast, NovH involved in novobiocin biosynthesis showed activity even in the absence of MbtH-like proteins, but its activity was stimulated by the presence of MbtH-like proteins.
Comparison of the active centers of CloH and NovH showed only one amino acid to be different, i.e. L383 versus M383. A site-directed mutagenesis of this amino acid in CloH (L383M) indeed resulted in an MbtH-independent mutant. All investigated tyrosine-adenylating enzymes exhibited remarkable promiscuity for MbtH-like proteins from different pathways and organisms. Additionally, the MbtH-like protein YbdZ from *E. coli* was found to co-purify with the heterologously expressed tyrosine-adenylating enzymes and to influence their biochemical properties markedly. Therefore, a knock-out strain was created in which the corresponding gene was deleted. This is of central importance for a reliable biochemical characterization of the tyrosine-adenylating enzymes.

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MEV005

KirCI and KirCII, the discrete acyltransferases involved in kirromycin biosynthesis

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Kirromycin is an antibiotic produced by *Streptomyces collinus* Tü 365. This compound binds to the elongation factor Tu (EF-Tu) and blocks bacterial protein biosynthesis. The molecule backbone is synthesized by a large complex of type I polyketide synthases and non-ribosomal peptide synthetases (PKS I/NRPS complex), encoded by the genes *kirAI-kirAVI* and *kirB* [1]. The PKSs KirAI-KirAV have a "*trans-AT*"-architecture. These megaenzymes have no acyltransferase domains integrated into the PKS modules. In contrast, KirAVI belongs to the classical "*cis-AT*"-type PKS, where the ATs are part of the PKS protein. In the gene cluster of kirromycin two separate genes, *kirCI* and *kirCII*, were identified, which are similar to acyltransferases.
To investigate the involvement of *kirCI* and *kirCII* in kirromycin biosynthesis, mutants were generated and analyzed for kirromycin production. The inactivation of *kirCI* (Δ*kirCI*) resulted in a significant reduction of kirromycin production. In Δ*kirCII* the kirromycin synthesis was completely abolished. To confirm the effects of the deletion of *kirCI* and *kirCII*, both mutants were complemented with the wild type genes. In the complemented strains the antibiotic production was restored to levels comparable with the parent strain *S. collinus* Tü 365. These data indicate that both genes are involved in kirromycin biosynthesis and the gene *kirCII* is essential for the production of this antibiotic.
For kirromycin assembly, a selective loading of ACPs with the building blocks malonyl-CoA and ethylmalonyl-CoA is required. To find out whether KirCI and KirCII are responsible for this precursor supply and to determine the substrate specificity of these enzymes, an *in vitro* ACP loading assay was carried out. Therefore KirCI, KirCII and two selected ACPs were expressed in *E. coli* and purified. The proteins were used in the *in vitro* assay and the loading of malonyl-CoA, methylmalonyl-CoA and ethylmalonyl-CoA to the ACPs was monitored by autoradiography and HPLC/ESI-MS. The experiments showed that KirCI loads specifically malonyl-CoA onto ACP4 and the second enzyme, KirCII, is the first biochemically characterized "*trans-AT*" with high specificity for ethylmalonyl-CoA and transfers this substrate to ACP5 [2]. Thus, there is a specific recognition of the ACP of module 4 and 5 by KirCI and KirCII, respectively. To our knowledge, such interaction mechanism, where a free-standing AT-protein that provide unusual building block, dock site-specific to the "recipient"-ACP to achieve structural diversity in polyketides was not characterized until now.

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MEV006

Investigation of the type II polyketide synthase from Gram-negative bacteria *Photorhabdus luminescence* TT01

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The aromatic heptaketide anthraquinone (AQ-256) is produced by the entomopathogenic Gram-negative bacterium *Photorhabdus lumicescence* TT01 (1). Previous studies have shown that the type II polyketide synthase (type II PKS) is responsible for the AQ-256 biosynthesis, because the typical octaketide shunt products known from actinorhodin biosynthesis could be identified (2). The gene cluster consists of ketosynthase (KS_α), chain length factor (CLF or KS_β), acyl-carrier protein (ACP), two cylases, one ketoreductase, one phosphopantetheinyl transferase (PPTase) and two proteins with possible function as a CoA ligase (AntG) and hydrolase (AntI), respectively.
In this study, we show that *E. coli* could be used as host for *in vivo* analysis of the biosynthesis by combining two Duet vectors including whole or partial gene cluster. Not only the shunt products could be identified by HPLC-MS, but also the function of the genes could be investigated in *E. coli*. Most proteins were expressed in soluble fraction in *E. coli* BL21 DE(3) and successfully purified. ACP could only be activated by the PPTase in company with AntG, but not by Sfp or MtaA. It looks as if the PPTase and AntG have strong interaction with each other. Site-directed mutants of AntG were generated and their activities could be tested. Additional disruptions of the gene *antG* and *antI* in TT01 were also performed. Utahmycin (3) was identified in the TT01 AntI knockout mutant. The hydrolase AntI was responsible for heptaketide formation from octaketide. Finally, *in vitro* experiments were performed leading to production of octaketide shunt products using the minimal PKS, KR and CYC/ARO.

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MEV007

Purification and Characterisation of the Flavin-Dependent Monodechloroaminopyrrolnitrin 3-Halogenase from Pyrrolnitrin Biosynthesis

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Pyrrolnitrin is an antifungal compound first isolated from *Pseudomonas pyrrocinia*. The gene cluster and the corresponding enzymes responsible for pyrrolnitrin biosynthesis were identified in *Pseudomonas fluorescens* (BL915) and other pyrrolnitrin producing bacteria. The third enzyme, monodechloroaminopyrrolnitrin (MCAP) 3-halogenase (PrnC), catalyses the regioselective chlorination of MCAP in the 3-position of the pyrrole ring. PrnC is a flavin-dependent halogenase and its reaction mechanism is suggested to be very similar to that of other flavin-dependent halogenases. However, the amino acid sequence shows hardly any similarity to the amino acid sequence of tryptophan halogenases for some of which three-dimensional structures are known.
Additionally, PrnC is, besides the tryptophan halogenases, the only known flavin-dependent halogenase that catalyses the halogenation of a free substrate. Other known flavin-dependent halogenases catalysing the chlorination of a pyrrole moiety act on a substrate bound to a peptidyl carrier protein making elucidation of the 3-D structure of these enzymes, especially in the presence of substrate, very difficult. Thus, the 3-D structure of PrnC is of high importance to understand how substrate specificity and regioselectivity are regulated in flavin-dependent halogenases.
So far, purification of PrnC in its active form has not been achieved satisfactorily which is partially due to the incompatibility of the tags used and the high tendency of PrnC to form aggregates with itself and other proteins. The lack of purified PrnC so far prevented further detailed analysis of the enzyme. Here we report a novel purification strategy leading to purified, active PrnC. Using the GST-fusion protein strategy it is possible to obtain homgeneous PrnC from recombinant *Escherichia coli* cells. The purity level of the eluted fusion protein highly depends on the growth temperature of the *E. coli* strain used for expression. MALDI-TOF-MS analysis revealed that the chaperonin GroEL and other proteins are co-purified by glutathione affinity chromatography when the growth temperature is not reduced to 20 °C. Dilution of the crude extract as well as the addition of Tween-20 has a high impact on the affinity of the fusion protein to the glutathione column. The addition of detergent does not inhibit halogenating activity, neither that of the fusion protein nor that of the purified PrnC after thrombin digestion.
Furthermore we can now report on first characterisation results giving first hints towards the reaction mechanism of PrnC.

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MEV008

EasG and FgaFS are key enzymes in the differentiation of ergot alkaloid biosynthesis in *Claviceps purpurea* and *Aspergillus fumigatus*

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Ergot alkaloids are secondary metabolites belonging to indole derivatives and are produced by a wide range of fungi with *Claviceps purpurea* as the most important producer for medical use. They show a broad spectrum of biological activities and their toxic effects were reported back to the middle ages. The early steps of ergot alkaloid biosynthesis are shared by *C. purpurea* and *Aspergillus fumigatus*, whereas later steps differ in the two fungi.[1] Chanoclavine-I aldehyde[2] was proposed as branch point for the biosynthesis in both fungi,[3] which is converted in *A. fumigatus* to the clavine-type alkaloid festuclavine by the festuclavine synthase FgaFS in the presence of the old yellow enzyme FgaOx3.[1] In *C. purpurea* chanoclavine-I aldehyde is converted to agroclavine by EasG in the presence of reduced glutathione without a requirement of EasA.[4] The enzymes were purified by affinity chromatography after overproduction in *E. coli* and characterized biochemically. The *in vitro* results for the formation of festuclavine catalysed by FgaOx3 and FgaFS proved two reduction steps. In contrast, agroclavine differs from festuclavine by a double bond between C8 and C9. Therefore only one reduction, but additionally an isomerisation step is necessary. We have shown that EasG was responsible for the reduction step and a non-enzymatic adduct with reduced glutathione for the isomerisation. The structures of festuclavine and agroclavine were unequivocally elucidated by NMR and MS analyses. In summary, EasG and FgaFS are the key enzymes controlling the branch point of ergot alkaloid biosynthesis in *C. purpurea* and *A. fumigatus*.

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MEV009

Biosynthesis of Cephlaosporin C Through Improved strains of *Aspergillus* and *Acremonium species*

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Antibiotics are secondary metabolites produced by microorganisms, extremely important to the health of our society. Cephalosporins are broad-spectrum antibiotics which are very similar in structure and action to penicillins but more resistant to β-lactamases. Optimization of media is useful to increase the production of antibiotics. Induction of mutation is commonly employed to increase the yield of secondary metabolites like antibiotics. Chemical mutation is preferred method because of the ease in handling and avoiding the hazardous effects of radiations.
Monitoring the concentrations of antibiotics and their precursors is required for their optimized production. Due to higher concentrations of proteins and other liquid phases in fermented broth aseptic sampling is a difficult task. Spectrophotometeric analysis can be used for the estimation of the antibiotic produced by microbes. Bioassay analysis can be done to confirm the antibiotic activity of the antibiotic produced. HPLC is used to differentiate the specific antibiotic from other secondary metabolites of microbes from the fermented broth.
The aim of this research work was to optimize media conditions and improvement of fungal strains through chemical mutation for enhanced Cephalosporin C (CPC) production by *Aspergillus* and *Acremonium* species.
For media optimization different concentrations of media contents were analyzed for increased production. Best results were shown by fermentation media supplemented with sucrose 30mg/ml. While DL-methionine shows optimum yield at 3mg/ml; and fermentation media supplemented with ammonium sulphate 7.5mg/ml as nitrogen sources gave maximum yield.
For mutation induction fungal strains were treated with 400μg/ml of Ethyl methane sulfonate (EMS) for 30-80 minutes. It was observed that time of chemical treatment was inversely proportional to the survival of fungal strains; minimum survival rate was obtained at treatment of 1 hour.
The mutants were then further analyzed for CPC production with optimized media conditions in similar way as done earlier before induction of mutation. Results obtained showed that very small increase in CPC production, in few fungal strains but not in all. This might be due to insufficient mutation for target genes (i.e., involved in CPC production) in remaining fungal species.
The spectrophotometeric and HPLC analysis of fermented broths were performed to analyze CPC yields. Similarities were observed in the results of both analyses. On spectrophotometeric and HPLC analysis, before mutation maximum yield of CPC (2.583 and 0. 254mg/ml respectively) was obtained with *Acremonium kiliense* FCBP # 162, respectively and after mutation maximum production of CPC (2.346, 0.24mg/ml respectively) was achieved with *Acremonium furcatum* FCBP # 409.
The bioassay analysis of the fungal strains and survivors (mutants) were performed to confirm antibacterial activity of CPC. The increased antibacterial activity was observed for some strains after mutation, for others it was decreased and it remained constant for remaining strains.

MEV010

Systems biology of the marine antibiotic producer *Phaeobacter gallaeciensis*

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Marine bacteria gain more interest since it is assumed that among these bacteria is a great potential of secondary metabolites which may be of industrial or medical interest. The *Roseobacter* clade is one of the most prevalent marine microorganisms, which are highly distributed in the oceans [1]. Many new species were found in the last few years and a rich repertoire of metabolic pathways has been identified. However, there is little information about the *in vivo* use in the marine environment [2, 3]. *Phaeobacter gallaeciensis* has the ability to produce a new interesting antibiotic, the tropoditiethic acid (TDA). Furthermore, other secondary metabolites, so called "Roseobacticides", were found, which inhibit the growth of diverse marine algae and bacteria [4, 5]. This makes the bacterium interesting for studies in systems biology, to develop optimization strategies and enhance the secondary metabolite production.
In this work *P. gallaeciensisis* investigated by systems wide metabolic flux analysis using ^{13}C-labelling studies and computational flux modelling with the software OpenFlux [6]. This provides a first insight into the *in vivo* use of its pathways. The first set of experiments focussed on the impact of different carbon sources. Together with transcriptome profiling this will

provide an insight into the regulation network in order to approach the complete picture of the cell.
First labelling studies showed that glucose is metabolized only via the Entner-Doudoroff pathway. This is an extremely unusual flux distribution and seems to be characteristic for the *Roseobacter* clade. In this study the impact of nutritional changes on the flux distribution was investigated by performing the first systems wide metabolic flux analyses.
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MEV011

Characterization and manipulation of the biosynthetic pathway of cyanobacterial tricyclic microviridins in E. coli

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Cyanobacteria are a structurally diverse group of bacteria, making a variety of biochemically active natural products using mostly the nonribosomal machinery of large multienzyme complexes. Microviridins are the largest known cyanobacterial oligopeptides synthesized through a unique ribosomal route (1). The unprecedented microviridin gene cluster encodes for a precursor peptide (MdnA), two novel ATP-grasp ligases (MdnB and C), a GNAT-type acetyltransferase (MdnD) and an ABC-transporter (MdnE).Microviridins comprise an unrivaled multicyclic cage-like architecture, carrying characteristic ω-ester and a secondary ω-amide bond. They are produced by different isolates of cyanobacteria, including the unicellular, bloom-forming freshwater cyanobacteriumMicrocystis aeruginosaNies843. The serine protease inhibitory activity contributes to both ecological and pharmacological relevance of microviridins. Here we report the construction of a stable expression platform for heterologous expression of microviridins inE. coli. Biostatistics and mutational analysis identified the conserved PFFARFL motif in the precursor peptide as a recognition sequence for the ATP-grasp ligases. Manipulations of the C-terminal part of the leader peptide abolished lactam ring formation of microviridins.The ABC-transporter MdnE was unveiled to be crucial for cyclization and processing of microviridins, probably holding and stabilizing a putative microviridin maturation complex at the inner membrane (2). Site-directed mutagenesis in the microviridin core sequence showed flexibility of the microviridin biosynthetic pathway to be used for peptide engineering. We determined residues that are important for the protease inhibition and are currently in process to optimize the product for different pharmaceutical targets. Furthermore, we developed a method to express cryptic microviridin precursor peptides from field and lab samples.
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MEV012

Evaluation of *Streptomyces coelicolor* as a heterologous expression host for natural products from marine filamentous cyanobacteria

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Filamentous marine cyanobacteria are rich sources of bioactive natural products and employ highly unusual biosynthetic enzymes in their assembly. However, the current lack of techniques for stable DNA transfer into these filamentous organisms combined with the absence of heterologous expression strategies for non-ribosomal cyanobacterial gene clusters prohibit the creation of mutant strains or the heterologous production of these cyanobacterial compounds in other bacteria. In this study, we evaluated the capability of a derivative of the model actinomycete *Streptomyces coelicolor* A3(2) to express enzymes involved in the biosynthesis of the protein kinase C activator lyngbyatoxin A from a Hawaiian strain of *Moorea producta* (previously classified as *Lyngbya majuscula*). Despite large differences in GC content between these two bacteria and the presence of multiple TTA/UUA leucine codons in lyngbyatoxin open reading frames, we were able to achieve expression of LtxB and LtxC in *S. coelicolor* M512 and confirmed the *in vitro* functionality of *S. coelicolor* overexpressed LtxC. Attempts to express the entire lyngbyatoxin A gene cluster in *S. coelicolor* M512 were not successful because of transcript termination observed for the *ltxA* gene, which encodes a large non-ribosomal peptide synthetase. However, these attempts did show a detectable level of cyanobacterial promoter recognition in *Streptomyces*. Successful *Streptomyces* expression of biosynthetic enzymes from marine cyanobacteria provides a new platform for biochemical investigation of these proteins and a promising avenue for combinatorial biosynthesis between these two bacterial phyla.

MEP001

Endophytic fungi, the microbial factories of associated plant secondary metabolites: Camptothecin as an example

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Endophytic fungi inhabit healthy tissues of plants and occasionally produce associated plant secondary metabolites [1-5]. We recently isolated an endophytic fungus, *Fusarium solani* from the bark of *Camptotheca acuminata*, which is capable of producing the anticancer pro-drug camptothecin (CPT) and two structural analogues in axenic monoculture [6]. We deciphered a cross-species biosynthetic pathway where the endophyte utilizes indigenous geraniol 10-hydroxylase, secologanin synthase, and tryptophan decarboxylase to biosynthesize CPT precursors. However, to complete CPT biosynthesis, it requires the host strictosidine synthase [7]. The fungal CPT biosynthetic genes destabilized *ex planta* over successive subculture generations. The seventh subculture predicted proteins exhibited reduced homologies to the original enzymes proving that such genomic instability leads to dysfunction at the amino acid level. The endophyte with an impaired CPT biosynthetic capability was artificially inoculated into the living host plants and then recovered after colonization. CPT biosynthesis could still not be restored [7]. We further discovered the survival strategy of this endophyte by identifying typical amino acid residues in the CPT-binding and catalytic domains of its topoisomerase I [8]. Recently, it was also revealed that chrysomelid beetles (*Kanarella unicolor*) feeds on the leaves of CPT-containing *N. nimmoniana* without any apparent adverse effect [9]. We thus envisage addressing the following open questions: why and how do endophytes produce plant bioactive compounds? What are the diverse interactions that endophytes have with other coexisting endophytes, host plants, insects, and specific herbivores? Elucidating these connections can not only enhance the understanding of evolution of complex defense mechanisms in plants and associated organisms, but also help in the sustained production of plant compounds using endophytes harbored within them.
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MEP002

Biochemical characterization of ectoine hydroxylases from extremophiles

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Ectoine and 5-hydroxyectoine are widely used by members of the *Bacteria* to offset the detrimental effects of high osmolarity on cellular physiology. Both compatible solutes also possess stabilizing effects for macromolecules and these properties, sometimes also referred to in the literature as "chemical chaperones", have spurred considerable biotechnological interest in ectoines. They have already found practical uses in cosmetics, skin-care products, as protein- and whole cell stabilizers and medical applications are currently envisioned as well. Ectoine synthesis is osmotically stimulated and catalyzed by the EctABC enzymes. A subset of the ectoine producers typically convert part of the newly produced ectoine into 5-hydoxyectoine through the enzymatic action of the EctD hydroxylase, a member of the non-heme iron (II) and 2-oxoglutarate-dependent deoxygenase super-family (1, 2). Although closely related in chemical structure, ectoine and 5-hydroxyectoine possess different properties, with 5-hydroxyectoine being often the more effective stabilizing compound and the more potent cellular stress protectant (3). Ectoine hydroxylases from *Virgibacillus salexigens* (1) and *Streptomyces coelicolor* (3) have been biochemically characterized and a high-resolution crystal structure of the EctD protein from *V. salexigens* has been solved (2). This crystal structure revealed the positioning of the iron ligand within the active site of the EctD enzyme but it contained neither the substrate ectoine nor the co-substrate 2-oxoglutarate. To advance our biochemical understanding of this enzyme and to characterize EctD-type proteins for further crystallographic studies, we have characterized the properties of ectoine hydroxylases from microorganisms that can colonize habitats with

extremes in salinity (*Halomonas elongata*), pH (*Alkalilimnicola ehrlichii*; *Acidiphilium cryptum*) or temperature (*Sphingopyxis alaskensis*; *Geobacillus* sp. Y412MC10). Although the kinetic parameters and catalytic properties of the characterized ectoine hydroxylases from these extremophiles are very similar, some of studied EctD proteins are very robust enzymes that makes them interesting candidates as catalyst in recombinant-DNA based whole-cell biotransformation processes and for structural analysis.

(1) Bursy, J., Pierik, A.J., Pica, N. and Bremer, E. (2007). J. Biol. Chem. 282:31147-31155.
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MEP003

Structure-guided site-directed mutagenesis of the ectoine hydroxylase from the moderate halophile *Virgibacillus salexigens*

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Increases in the external salinity triggers water efflux from the microbial cell and the ensuing dehydration of the cytoplasm negatively affects cell growth and impairs survival. To balance the osmotic gradient across the cytoplasmic membrane, many microorganisms amass a selected class of organic compounds, the "compatible solutes". One of the most widely used compatible solutes by members of the *Bacteria* is the tetrahydropyrimidine ectoine and its derivative 5-hydroxyectoine. These two compatible solutes have attracted considerable biotechnological attention, are produced in large-scale fermentation processes employing halotolerant microorganisms and are commercially used in skin-care products, as protein and cell stabilizers and medical applications of ectoines are envisioned. About a third of all microbial ectoine producers also synthesize 5-hydroxyectoine from ectoine. 5-hydroxyectoine is synthesized by a stereo-specific hydroxylase (EctD) that is a member of the non-heme iron (II) and 2-oxoglutarate-dependent dioxygenase super-family (1). Microbial EctD-type proteins are closely related to each other and belong structurally to the PhyH-subgroup within the dioxygenase super-family. This was disclosed by the recently reported high-resolution crystal structure of the ectoine hydroxylase from the moderate halophile *Virgibacillus salexigens* (2). This structure revealed the unambiguous positioning of the iron ligand within the active site of the EctD enzyme by an evolutionarily conserved iron-binding motif, the so-called 2-His-1-carboxylase facial triad. However, the obtained crystal structure contained neither the substrate ectoine nor the co-substrate 2-oxoglutarate. Here we used the crystal structure of the *V. salexigens* EctD enzyme as a template to functionally probe, via site-directed mutagenesis, amino acid residues that seemed important for the correct positioning of the ligand ectoine and the co-substrate 2-oxoglutate with respect to the catalytically critical iron-ligand. These studies allowed us to map the spatial organization of the active site of EctD that is buried in a deep caveat formed by the monomeric EctD protein. A detailed reaction scheme for the stereo-chemical hydroxylation of ectoine to 5-hydroxyectoine catalyzed by the EctD enzyme will be presented.

(1) Bursy, J., Pierik, A.J., Pica, N. and Bremer, E. (2007). J. Biol. Chem. 282:31147-31155.
(2) Reuter, K. Pittelkow, M., Bursy, J., Heine, A., Craan, T. and Bremer, E. (2010). PLoS ONE 5(5):e10647

MEP004

Biosynthesis, Partial Purification and Characterization of Invertase from *Sacchromyces cerevisae* by Solid-State Fermentation of Carrot Peels

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Potential of different *Sacchromyces* species,cultivated under solid-state fermentation (SSF) using carrot peels (*Daucus carota* L.) as substrate was investigated. The highest productivity of invertase (7.95 U mL^{-1}) was achieved by using *Sacchromyces cerevisae* on 90% initial moisture content with 2.5 ml inoculum size after 72 h of incubation period. The enzyme was purified about 1.42 fold by ammonium sulphate precipitation. It showed thermal stability from 20-40° C over a pH range 5.5 to 6.5 with maximum activity at pH 5.5 and 50° C. The enzyme was highly active towards sucrose at both concentrations viz: 0.1 M and 0.5 M, but it showed less activity towards glycerol. It was completely inhibited by Hg^{2+} (1mM) and slightly stimulated by Co^{2+} and Na^{+1}at the same concentration.

MEP005

Biosynthesis of the iron-guanylylpyridinol cofactor of [Fe]-hydrogenase in methanogenic archaea as elucidated by stable-isotope labeling

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[Fe]-hydrogenase catalyzes the reversible hydride transfer from H_2 to methenyltetrahydromethanoptherin, which is an intermediate in methane formation from H_2 and CO_2 in methanogenic archaea. The enzyme harbors a unique active site iron-guanylylpyridinol (FeGP) cofactor, in which a low-spin Fe^{II} is coordinated by a pyridinol nitrogen, an acyl group, two carbon monoxide, and the sulfur of the enzyme's cysteine. Here, we studied the biosynthesis of the FeGP cofactor by following the incorporation of ^{13}C and ^{2}H from labeled precursors into the cofactor by growing methanogenic archaea and by subsequent NMR, MALDI-TOF-MS and/or ESI-FT-ICR-MS analysis [s1] of the isolated cofactor and reference compounds. The cofactors pyridinol moiety was found to be synthesized from three C-1 of acetate, two C-2 of acetate, two C-1 of pyruvate, one carbon from the methyl group of l-methionine, and one carbon directly from CO_2. The metabolic origin of the two CO- ligands was CO_2 rather than C-1 or C-2 of acetate or pyruvate excluding that the two CO are derived from dehydroglycine as has previously been shown for the CO-ligands in [FeFe]-hydrogenases. A formation of the CO from CO_2 via direct reduction catalyzed by a nickel-dependent CO dehydrogenase or from formate could also be excluded. When the cells were grown in the presence of ^{13}CO the two CO-ligands and the acyl group became ^{13}C labeled, indicating that free CO is either an intermediate in their synthesis or that free CO can exchange with these iron-bound ligands. Based on these findings, we propose pathways of how the FeGP cofactor might be synthesized.

MEP006

A recombinant system for the biotransfomation of ectoine into the chemical chaperone 5-hydroxyectoine

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Ectoine and 5-hydroxyectoine are an important class of compatible solutes that are synthesized by many microorganisms in response to high salinity. Some ectoine producers transform part of the newly formed ectoine into 5-hydroxyectoine through the enzymatic action of the ectoine hydroxylase (EctD), a non-heme iron (II)- and 2-oxoglutarate dependent dioxygenase (1, 2). Ectoine and 5-hydroxyectoine have attracted considerably biotechnological interest since they possess interesting stabilizing properties for proteins, nucleic acids, membranes and whole cells. Although closely related in chemical structure, ectoine and 5-hydroxyectoine have different properties, with 5-hydroxyectoine being often the more effective stabilizing compound and the more potent cellular stress protectant. Currently, ectoine and 5-hydroxyectoine are biotechnologically produced by large-scale fermentation of halotolerant microorganisms using the bacterial milking process. Synthesis of 5-hydroxyectoine depends on the prior production of ectoine, a process whose efficiency depends on various environmental conditions and the growth phase of the culture. As a consequence, ectoine/5-hydroxyectoine producers often contain a mixture of these compounds and this requires time-consuming and costly separation procedures during the downstream processes for the biotechnological production of pure ectoine and 5-hydroxyectoine. Recombinant-DNA based biotransformation processes might be an interesting alternative to produce 5-hydroxyectoine. *Escherichia coli* can import ectoine under osmotic stress conditions (via the ProP and ProU transporters) but it cannot synthesize it. We set up a cell factory of an *E. coli* strain that is unable to synthesize its natural compatible solute trehalose and that carries on a plasmid heterologous *ectD* genes whose expression can be triggered by adding an inducer to the growth medium. This biotransformation process was optimized by using different expression strains, various cultivation conditions and by employing EctD proteins from various extremophiles. We found that ectoine is effectively taken up by these recombinant *E. coli* cells, converted efficiently into 5-hydroxyectoine and that a substantial portion of the newly produced 5-hydroxyectoine is secreted into the growth medium.

(1)Bursy, J., Pierik, A.J., Pica, N. and Bremer, E.(2007) Osmotically induced synthesis of the compatible solute hydroxyectoine is mediated by an evolutionarily conserved ectoine hydroxylase. J. Biol. Chem.282:31147-31155.
(2)Reuter, K., Pittelkow, M., Bursy, J., Heine, A., Craan, T. and Bremer, E.(2010) Synthesis of 5-hydroxyectoine from ectoine: crystal structure of the non-heme iron (II) and 2-oxoglutarate-dependent dioxygenase EctD. PLoS ONE 5(5):e10647.

MEP007

Identification and toxigenic potential of a cyanobacterial strain (*nostoc* sp.)

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Cyanobacteria are well known for their production of a multitude of highly toxic and / or allelopathic compounds. These products are peptides, depsipeptides or alkaloids. Among the photosynthetic microorganisms, cyanobacteria belonging to the genus *Nostoc* are regarded as good candidates for producing biologically active secondary metabolites, which are highly toxic to humans and other animals.
The current scenario of toxicity has become more and more threatening and importance in recent years due to increase in the rate of deaths in animals especially canine and cows. Toxin-producing cyanobacteria represent a health hazard, and can cause death, mainly from liver damage, upon ingestion of drinking water infested with cyanobacterial supplement products.
This prompted us to do an endeavor towards to molecular detection of toxins, microcystin, anatoxin- a, and other bioactive compounds by PCR and LC-MS, in order to introducing the probably causative compound in incidents of fatal canine.
Our molecular data, demonstrate that the studied strain contains *nosF* gene and most likely products of unusual amino acid 4-methylproline. In addition to validating the use of eight oligonucleotide primers set for identification of potential of toxin/ bioactive compounds in *Nostoc* strain, this study also defines some chemical analyses, that will be useful as probes for future studies of the synthesis of natural products in that strain.
Result of ion chromatograms and MS^2 fragmentation patterns showed that, while, there were three different peptidic compound classes (anabaenopeptin, cryptophycin and nostocyclopeptides), there were not any sign from the presence of anatoxin- a, homoanatoxin-a, hassallidin and microcystin in that strain. Moreover, the biochemical assays have aimed to detection of the presence of antifungal effects in cell extract. The phylogeny of the strain was also investigated by combination genetic and phenotypic relationships of the *Nostoc* strain.
In spite of presence these compounds, especially the depsipeptides cryptophycins, with strong cytotoxic effect on the tubulin polymerization, there is no evidence of overt neurotoxicity or histopathological changes indicative of effects on the brain and peripheral nerves were reported in the dogs or rats.
The above findings indicate that cyanobacteria are a promising but still unexplored natural resource possessing many bioactive compounds useful for the pharmaceutical, food and cosmetic industry. Of the new drugs approved between 1983 and 1994, up to 80% of antibacterial and anticancer drugs were derived from natural products. Indeed, bioactive compounds of algae are of special interest in the development of new environment harmless. The present study aims the preliminary investigation of antimicrobial and toxicity evaluation of *Nostoc*. This merits further and more detailed investigations.

MEP008

Molecular mechanisms of rhamnolipid synthesis in *Pseudomonas aeruginosa* during batch fermentation

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Pseudomonas aeruginosa is a gram-negative, opportunistic human pathogen that produces the biosurfactant rhamnolipid amongst others as secondary metabolites during stationary growth phase. The regulation of rhamnolipid synthesis is tightly governed by a complex regulatory network including bacterial quorum sensing systems as well as different sigma factors. Production of rhamnolipids is hence not solely dependent on cell density but also nutrient availability and stress. The genes for mono- and di-rhamnolipid synthesis, rhamnosyltransferases 1 and 2 respectively are encoded in one mutual operon which is under the direct control of the Rhl-quorum-sensing system and stationary phase sigma factor RpoS. The Rhl-quorum-sensing system in turn is controlled by the Las quorum-sensing system and nitrogen limitation sigma factor RpoN. Additional fine-tuning of the regulatory network is achieved by various external negative and positive regulators.
Production of rhamnolipids by *Pseudomonas aeruginosa* PAO1 during batch fermentation under nitrogen limitation with sunflower oil as carbon source was recently demonstrated [1] and the production capacity has been evaluated [2]. However, the molecular regulatory network during rhamnolipid batch fermentation is not yet fully elucidated on molecular regulation level.
In this study we present gene expression data of the relevant systems involved in the regulation of rhamnolipid production during small-scale batch cultivation under different medium compositions and nutrient supplies using SYBR Green mediated quantitative real-time PCR. Furthermore, the gene expression during the time course of a standard 30L-batch fermentation is monitored.
The aim of this project is the optimisation of rhamnolipid production under large-scale conditions for commercial production processes. Full comprehension of the molecular regulatory mechanisms behind rhamnolipid synthesis is the key to manipulating and improving the rhamnolipid production capacities.

1. Muller, M.M., et al.,Pseudomonas aeruginosa PAO1 as a model for rhamnolipid production in bioreactor systems.Appl Microbiol Biotechnol, 2010.87(1): p. 167-74.
2. Muller, M.M., et al.,Evaluation of rhamnolipid production capacity of Pseudomonas aeruginosa PAO1 in comparison to the rhamnolipid over-producer strains DSM 7108 and DSM 2874.Appl Microbiol Biotechnol, 2011.89(3): p. 585-92.

MEP009

Characterization of an antimicrobial substance produced by *Bacillus pseudomycoides* DSM 12442

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Lantibiotics are lanthionine containing antimicrobial peptides. Lantibiotics possess structural genes which encode inactive prepeptides. During maturation, the prepeptide undergoes posttranslational modifications including the introduction of rare amino acids as lanthionine and methyllanthione as well as the proteolytic removal of the leader. The gene cluster includes structural (*lanA*) and other genes which are involved in lantibiotic modification (*lanM,lanB, lanC,lanP*), regulation (*lanR,*lanK), export (*lanT (P)*) and immunity (*lanEFG*).
Genomic data mining showed a new complete lantibiotic gene cluster in the Gram-positive bacterium *Bacillus pseudomycoides*DSM 12442. An antimicrobial activity was detected only in an isopropanol extract of the cell pellet but not in the culture supernatant. In agar well diffusion assays, it showed activity against many Gram-positive bacteria, including bacilli, streptococci and staphylococci, whereas no activity was observed against Gram-negative bacteria. The antimicrobial substance was relatively stable at high temperature ($\leq 100\ ^0C$), low pH (< 7) and in organic solvents (e.g. acetone, ethanol, etc.). The partially purified substance was predicted to have a mass of 2786.59 Da by MALDI-TOF analyis.
To demonstrate the connection between the lantibiotic gene cluster and the antimicrobial activity,in vitrostudies and heterologous expression of*lanA*and*lanM*were conducted. Clones of both genes were constructed. So far, the LanM has been successfully expressed and purified. LanA expression and purification is under progress. A factor Xa cleavage site was introduced into LanA, so that the leader peptide can be removed from the modified peptide to investigate its biological activity.

MEP010

Heterologous expression of synthetic lantibiotic libraries in *S. carnosus*

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Many gram-positive bacteria produce short peptides with antimicrobial activity - so called “lantibiotics”. They are characterized by unusual amino acids and lanthionine rings that are both introduced by posttranslational modifications. Lantibiotics primary act by binding to the cell wall precursor Lipid II, thus inducing pores in the cytoplasmic membrane of other gram-positive bacteria. All lantibiotics are synthesized as inactive precursors and subsequently activated through proteolytic cleavage by specific proteases. The type A lantibiotic gallidermin, produced by *Staphylococcus gallinarum*, is considered for the treatment of acne (*Propionibacterium acnes*) and staphylococcal infections like mastitis.
We introduced the relevant biosynthesis genes *gdmBCDHTQ* and the structural gene *gdmA* on separate plasmids in *S. carnosus* TM300. By using bioactivity assays as well as HPLC- and MS-analysis, we demonstrated that the modified *S. carnosus* is able to produce the gallidermin precursor that can be activated by the specific protease GdmP.
This two-plasmid expression system is now used as a tool for the expression of a synthetic *gdmA*-library in order to identify improved gallidermin-derivatives. In a similar approach, other lantibiotics such as nisin can be produced in *S. carnosus*. With this efficient system, we expect to produce and identify a high variety of novel lantibiotics.

MEP011

Development of Fed-Batch Strategies for Antibiotic Production of *Actinoplanes friuliensis*

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Actinoplanes friuliensis, a rare actinomycete, is the producer strain of friulimicin, a lipopeptide antibiotic which is active against a broad range of multiresistant gram-positive bacteria such as methicillin-resistant *Enterococcus spec*. and *Staphylococcus aureus* (MRE, MRSA) strains (Aretz, 2000).
In order to improve the understanding of the complex metabolic network of the friulimicin biosynthesis in *A. friuliensis*, a genome-scale network model will be developed and characterized (Insilico Biotechnology). To validate the model and to perform metabolic flux analysis, data from cultivations of *A. friuliensis* are collected and applied to this model. The cultivations are carried out in a bioreactor under defined and controlled conditions. A chemically defined production medium, especially developed for *A. friuliensis*, is used. This defined medium is a prerequisite for the quantitative analysis of cell metabolism during the cultivations and is also necessary to verify a new developed middle infrared spectroscopy method (AquaSpec Technology, micro-biolytics GmbH). With this method, all known substrates and metabolites can be measured in one sample.
By developing fed-batch cultivation strategies, the production of the friulimicin inhibiting by-product ammonium could be prevented.
The validated flux model, combined with data of cultivation and transcription analysis, will subsequently give hints for directed genetic modifications and optimization of process control strategies with the objective to redirect metabolic fluxes towards friulimicin production.

Aretz, W.; Meiwes, J.; Seibert, G.; Vobis, G.; Wink, J., J Antibiot (Tokyo), 2000, 53, 807-815.7

MEP012

The catalytic and regulatory role of aconitase AcnA in Streptomyces viridochromogenes Tü494.

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In many organisms, aconitases have dual functions: they serve as primary metabolisms enzymes in the tricarboxylic acide cycle and as regulators of iron metabolism and oxidative stress response. Inactivation of the aconitase AcnA in Streptomyces viridochromogenes Tü494, the producer of herbicide antibiotic phosphinothricyl-alanyl-alanin (phosphinothricin tripeptide=PTT), leads to strong defects in physiological and morphological differentiation. This mutant (MacnA) fails in sporulation and antibiotic production which are characteristic secondary metabolism specific properties of sreptomyces. Furthemore, AcnA, in addition to its catalytic function, is capable of binding to iron responsive elements (IREs) thus altering the m-RNA stability in a similar mechanism described for the iron regulatory proteins (IRPs). A mutation preventing the formation of the [4Fe-4S] cluster of the aconitase (HisacnA1(C538A)) abolishes its catalytic activity, but does not inhibit its RNA-binding ability. In contrast, HisacnA2(Δ125-129)in which 5 highly conserved aminoacids of AcnA are deleted shows an higher affinity to IREs than HisacnA. Furthermore, expression of HisacnA2 (Δ125-129) instead of native acnA gene results in a strain that sporulates earlier and has increaseg PTT production than wild type. This correlates with the improved RNA-binding ability of HisacnA2(Δ125-129). In silico analysis of the S. viridochromogenes genome revealed several IRE-like structures e.g. upstream of recA gene, involved in the bacterial SOS response, ftsZ gene, required for the onset of sporulation in streptomyces. The binding of AcnA to these IREs is confirmed in gel shift assays. In conclusion, the demonstrated regulatory function of AcnA on the posttranscriptional level provides a new, so far unknown and unexploited form of regulation of secondary metabolism in streptomyces which might serve as possibility to optimize antibiotic production.

MEP013

Metabolic engineering of *Corynebacterium glutamicum* for the production of β-alanine

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β-alanine is commercially available as a nutrition supplement for athletes and is a possible intermediate for the fermentative production of acrylic acid. Here, we report about the metabolic engineering of *Corynebacterium glutamicum* for the production of β-alanine. Biomass formation and growth rate of *C. glutamicum* cultivated in glucose minimal media were not altered by supplementation with up to 200 mM β-alanine. Production of β-alanine with *C. glutamicum* was achieved by overexpression of the aspartate 1-decarboxylase gene *panD*. During growth in glucose minimal media β-alanine accumulated in the culture supernatant of cells overexpressing *panD*, but not of the empty vector control strains. To enhance production of β-alanine the *panBC*-operon coding for 3-methyl-2-oxobutanoate hydroxymethyltransferase and pantoate-β-alanine ligase was deleted in *C. glutamicum* to avoid the drain of β-alanine into the pantothenate/Coenzyme A-pathway, thereby causing a pantothenate auxotrophy. Deletion of *panBC* in *C. glutamicum* R127 led to a 12 % increase of β-alanine production. However, supplementation of the auxotrophic strain with less than 3 µM pantothenate resulted in decreased biomass formation and favored production of α-alanine over β-alanine. α-alanine occurred as a byproduct in all production experiments. To lower the byproduct formation the gene *alaT* coding for the main α-alanine-synthesizing transaminase in *C. glutamicum* was deleted in combination with *panBC*. The resulting so far most promising strain *C. glutamicum* ATCC13032Δ*panBC*Δ*alaT*(pVWEx1-*panD*) produced 20 mM β-alanine and 2 mM α-alanine as byproduct from CGXII media with 4 % glucose as carbon and energy source.

MEP014

Secondary metabolites of fungi from the German Wadden Sea

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The Wadden Sea forms an interesting habitat since it underlies permanent changes due to the tidal influence. Fungi living in such an environment presumably need a high metabolic versatility in order to survive. Because metabolic versatility also may relate to secondary metabolite biosynthesis, fungal strains isolated from the German Wadden Sea were investigated with regard to secondary metabolite production. The 109 strains isolated from sediments were grown under varying culture conditions, in shaken or static cultures and in different media. Cultures were extracted applying liquid-liquid extraction, and extracts were analysed by HPLC-DAD/MS. The results displayed a strong influence of the media composition on metabolite production. One of the fungal strains showed exceptionally attractive metabolite profiles and was selected for detailed investigations. The structures of several of the purified compounds of this strain were identified by NMR spectroscopy as the known substances tricinonoic acid (Bashyal and Gunatilaka, 2010), 6-hydroxymellein, 6-methoxymellein (Dunn et al. 1979), orbuticin, 32-hydroxyorbuticin, antibiotic 15G256α-2, 15G256β-2, and 15G256π (Schlingmann et al. 2002). More importantly, six new compounds were elucidated in structure and bioactivity assays of these substances exhibited antibacterial and cytotoxic properties with the potential of possible biotechnological application.

Bashyal, B.P., Gunatilaka, A.A.L. (2010). Tricinonoic acid and tricindiol, two new irregular sesquiterpenes from an endophytic strain of *Fusarium tricinctum*. Nat. Prod. Res. 24: 349-356
Dunn, A.W., Johnstone, R.A.W., King, T.J.,Lessinger, L., Sklarz, B. (1979). Fungal Metabolites. Part 7. Structures of C_{25} Compounds from *Aspergillus variecolor*. J.C.S. Perkin I: 2113-2117
Schlingmann, G., Milne, L., Carter, G.T. (2002). Isolation and identification of antifungal polyesters from the marine fungus *Hypoxylon oceanicum* LL-15G256. Tetrahedron 58: 6825-6835

MEP015

Terpenoids from *Corynebacterium glutamicum*

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Terpenoids are the most diverse class of natural products comprising more than 40,000 of structurally different compounds. They naturally occur in microbes, animals and a wide range of plant species, where terpenes often are produced as secondary metabolites. Terpenoids exert a huge variety of biochemical properties and physiological functions. Therefore their commercial applicability is not fully explored. At present terpenoid products are used in cancer therapy, treatment of infectious diseases, crop protection, food additives, flavors and cosmetics, but the large-scale chemical synthesis is often difficult or costly due to their structural complexity and the isolation from the natural sources usually does not yield the desired quantities. For that reason the microbial biosynthesis is a promising approach for the production. Moreover, all terpenoids derive from the same universal precursor molecule isopenthenyl pyrophosphate (IPP) or its isomer dimethylallyl pyrophosphate (DMPP).
In this work, the bacterium *Corynebacterium glutamicum* is analysed with respect to the production of carotenoids, terpene pigments of great commercial interest. The Gram positive *C. glutamicum* is used for the annual production of more than 3,000,000 tons of amino acids. The predominant carotenoids in *C. glutamicum* are C_{50}-terpene decaprenoxanthin and its glucoside. The yellow pigmented *C. glutamicum* possesses a carotenogenic gene cluster for the complete pathway of decaprenoxanthin synthesis starting from the precursors IPP and DMPP. A series of single gene deletions verified the proposed pathway leading to decaprenoxanthin as the respective precursor carotenoids accumulated which sometimes resulted in a changed cell color. Overexpression of *dxs*, encoding 1-deoxy-D-xylulose-5-phosphat synthase, the first enzyme of the endogenous non-mevalonate pathway, slightly enhanced accumulation of lycopene in Δ-*crtXYXYX* mutant. The possible influence of accumulating

various carotenoids instead of decaprenoxanthin on the growth behavior, sensitivity towards UV or oxidants will be assessed.

MEP016

Determination of influencing factors on mycotoxin production in *Alternaria alternata*

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Black-moulds of the genus *Alternaria* contaminate many foodstuffs and agricultural products. In addition to the economical damage these fungi can produce harmful secondary metabolites, the *Alternaria* toxins. Some of these mycotoxins such as alternariol (AOH), alternariolmonomethylether (AME), altenuene (ALT) and tenuazonic acid (TA) have been described as cytotoxic, genotoxic and mutagenic *in vivo* and *in vitro*. These mycotoxins were detected in many foodstuffs even under refrigeration conditions. To minimize the health risks of the consumers it is absolutely essential to determine factors which influence mycotoxin production of *Alternaria alternata*.

For the determination of influencing parameters a robust and reliable platform process was developed[1]. The system proofed to be highly reproducible and set the conditions for the monitoring of substrate consumption and mycotoxin production. Additionally, variation of single process parameters was possible. The influences of carbon and nitrogen source[2], aeration rate[1] and pH value were examined. By the choice of carbon and nitrogen source mycotoxin concentration and composition can be altered whereas due to the variation of aeration rate and pH value over a broad range optimum curves can be obtained. This study provides essential data to elucidate mycotoxin production in *Alternaria alternata*.

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MEP017

Production of cytotoxic tryprostatin B analogues by using the prenyltransferase FtmPT1

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The prenyltransferase FtmPT1 from *Aspergillus fumigatus* is involved in the biosynthesis of verruculogen [1]. This enzyme catalyzes the regular prenylation of cyclo-L-Trp-L-Pro (brevianamide F) of the indole nucleus at C-2 position, resulting in the formation of tryprostatin B, which was reported to be active as a cell cycle inhibitor [2;3]. It has been shown that FtmPT1 accepted, in addition to its natural substrate brevianamid F, seven other tryptophan-containing cyclic dipeptides [2;4].

In this study fourteen tryptophan-containing cyclic dipeptides, including all the four diastereomers of cyclo-Trp-Pro and cyclo-Trp-Ala, were converted to their C2-prenylated derivatives by using the overproduced and purified FtmPT1. The enzyme products were isolated on HPLC in preparative scales and their structures were elucidated by NMR and MS analyses. The cytotoxic effects of the produced compounds were tested with several human cell lines. The prenylated products showed significantly higher cytotoxicity against these cell lines than the respective non-prenylated cyclic dipeptides. Therefore we provided additional evidence that the prenylation is essential for the biological activity of tryprostatin analogues [5].

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MEP018

Identification of PyrG1 as a glycosyltransferase involved in the biosynthesis of pyrroindomycins

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Streptomyces rugosporus LL-42D005 produces pyrroindomycin A and its chlorinated derivative, pyrroindomycin B [1]. Pyrroindomycins are active against Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* strains [2]. Pyrroindomycins are related to other compounds containing a tetramic or tetronic acid moiety spiro-linked to a cyclohexene ring.

Little is known about the biosynthesis of pyrroindomycins. In pyrroindomycin B biosynthesis PyrH, a $FADH_2$-dependent tryptophan 5-halogenase, chlorinates tryptophan to yield 5-Cl-tryptophan the first intermediate in the biosynthesis of a three-ring pyrroloindole structure. No further information about the biosynthesis of pyrroindomycin B is available. We cloned around 30 kb of the pyrroindomycin biosynthetic gene cluster and we proposed the function of the ORFs we found. In order to obtain information about the function of these putative genes, inactivation experiments were performed. A putative glycosyltransferase gene (*pyrG1*) was identified and a deletion mutant was constructed. The resultant mutant strain *Streptomyces rugosporus* Δ*pyrG1* neither produces pyrroindomycin A nor pyrroindomycin B anymore. Instead, a new main compound with no pyrroindomycin UV-spectrum was detected. Isolation, purification and structure elucidation of the accumulated product allowed the characterisation of this compound as the aglycon of the polyketide moiety of pyrroindomycin A and B and provides first insight into the pyrroindomycin biosynthetic pathway.

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MEP019

Prenylation of hydroxynaphthalenes and flavonoids by indole prenyltransferases from fungi

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Fungal indole prenyltransferases of the dimethylallyltryptophan synthase (DMATS) superfamily are involved in the biosynthesis of prenylated indole alkaloids, and catalyze the prenylation of diverse indole derivatives.(1) These enzymes share no sequence, but structure similarity with the prenyltransferases of the CloQ/NphB group, which accepted hydroxynaphthalenes, 4-hydroxyphenylpyruvate, phenazine and flavonoids as substrates. We have demonstrated that some indole prenyltransferases accepted also hydroxynaphthalenes and flavonoids as substrates.(2,3) Nine prenylated flavonoids and twenty prenylated hydroxynaphthalenes have been isolated, and their structures were elucidated by MS and NMR analyses. It has been shown that, for an accepted hydroxynaphthalene, different enzymes produced usually the same major prenylated product, *i.e.* with a regular *C*-prenyl moiety at *para*- or *ortho*- position to a hydroxyl group. For hydroxynaphthalenes with low conversion rates and regioselectivity, *O*-prenylated and diprenylated derivatives were also identified as enzyme products. For flavonoids accepted by 7-DMATS, C-6 between two hydroxyl groups was the favorable prenylation position. The K_M values and turnover numbers (k_{cat}) of some prenyltransferases towards selected hydroxynaphthalenes, are comparable to those obtained by using indole derivatives. These results expand the potential usage of prenyltransferases of the DMATS superfamily as catalysts for chemical synthesis, and meanwhile, increase the structural diversity of prenylated compounds.

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MEP020

Ergot alkaloid gene cluster in the fungal family of Arthrodermataceae

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Ergot alkaloids play an important role as pharmaceuticals as well as toxins in food and feed industry.[1;2] Ergot alkaloids with a characteristic tetracyclic ergoline ring can be divided into three groups: clavine-type alkaloids, ergoamides and ergopeptines.[1] Comparison of the gene cluster for ergopeptines from *Claviceps purpurea* with those for clavine-type alkaloids from *Aspergillus fumigatus* and *Penicillium commune* revealed the presence of seven orthologous/homologous genes, which were speculated to be responsible for the formation of the ergoline system. Blasting genome sequences of different fungi with enzymes for ergot alkaloid biosynthesis, led to the identification of a putative ergot alkaloid gene cluster in fungi of the family Arthrodermataceae. The gene cluster consists of five genes with clear sequence similarity to those assigned to the early common steps of the ergot alkaloid biosynthesis, *i.e.* from prenylation of tryptophan to formation of chanoclavine-I aldehyde, a branch point for clavine-type ergot alkaloid and ergopeptine biosynthesis. The homologous genes being responsible for the conversion of chanoclavine-I aldehyde, *i.e. fgaOx3* and *fgaFS* in *A. fumigatus*[3] or *easG* in *C. purpurea*[4], were not found in arthrodermataceous fungi, nor further genes in the biosynthesis of later special steps in both fungi.

The function of one gene ChaDH, coding a chanoclavine-I dehydrogenase, was proven by gene cloning, expression and biochemical characterization of the overproduced enzyme. NMR and MS analyses of the isolated

enzyme product proved unequivocally ChaDH as NAD-dependent chanoclavine-I dehydrogenase like its homologue FgaDH.[5]

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MEP021

The interlocking between primary and secondary metabolism in the biosynthesis of the glycopeptide antibiotic Balhimycin

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Balhimycin is a glycopeptide antibiotic of vancomycin-type. Such antibiotics are used for the treatment of serious infections caused by multi-resistant gram-positive bacteria. To antagonize the consistently increasing number of the antibiotic resistance, it is important to understand the biosynthetic pathway of antibiotic production in details to optimize its production and advance its impact.
As glycopeptide balhimycin consists of a glycosylated heptapeptide backbone. Five of these seven amino acids derive from the shikimate pathway. The analysis of the gene cluster showed that in addition to the genes encoding the biosynthetic enzymes, the balhimycin gene cluster includes two genes (dahp, pdh) which encode the homologous key enzymes of the shikimate pathway. The previous research showed that the deletion and over expression of these additional genes inA. balhimycinaaffects the antibiotic production. The over expression ofdahpfrom the antibiotic gene cluster causes increased production of balhimycin. The deletion of the same gene causes the decreased antibiotic production. In contrast the over expression ofpdhfrom the balhimycin biosynthetic gene cluster leads to the lower antibiotic production and its deletion does not show any remarkable effects considering the antibiotic production. This fact could be explained by cross-regulation between tyrosine and phenyalanine biosynthetic pathway which was described forA. methanolica. ByA. methanolicatyrosine functions as an activator for prephenate dehydratase (Pdt) which catalyzes the first step reaction on the branching point from prephenate direction phenylalanine. Otherwise Pdt is feedback inhibited by phenylalanine. The overexpression of Pdt inA. balhimycinain the current work resulted in the increased antibiotic production what would explain the results of the previous research and confirm the similar regulation mechanism byA. balhimycinaandA. methanolicaon the branching point between tyrosine and phenyalanine biosynthesis.
The other disputable question in the tyrosine biosynthesis is the substrate specificity of Pdh. In-silico analysis let to assume that Streptomyce's Pdh is L-arogenate and not prephenate specific. The overexpression and purification ofA. balhimycinaPdh which was used in enzyme assay showed the prephenate specificity. The proof for L-arogenate specificity of Pdh fromA. balhimycinain an enzymassay has to be done.

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MEP022

Identification of a phenazine gene cluster in *Dermacoccus* sp. MT1.2, isolated from a Mariana Trench sediment

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A sediment sample was taken from the Mariana Trench at the third deepest point of earth, the Challenger Deep (10,898 m), in the western Pacific Ocean (11°19'911" N; 142°12'372" E) on 21 May 1998 by the remotely operated submersible Kaiko, using sterilized mud samplers during dive number 74. The sediment sample (approximately 2 ml) was stored at -20°C until analyzed for actinomycetes. 38 actinomycetes were isolated using marine and raffinose-histidine agar, and were characterized by phylogenetic analysis on 16S rRNA gene sequencing [1]. The strains were assigned to the genera *Dermacoccus* (19 isolates), *Kocuria* (1 isolate), *Micromonospora* (1 isolate), *Streptomyces* (5 isolates), *Tsukamurella* (11 isolates) and *Williamsia* (1 isolate).
The *Dermacoccus* isolates showed unusual secondary metabolite profiles determined by HPLC-DAD analysis. Strains MT1.1 and MT1.2 exhibited the highest productivity and were therefore selected for fermentation studies using ISP2 and 410 media, respectively. This led to the production of seven novel phenazine metabolites, the dermacozines. Structure elucidation was performed by ^{13}C and ^{1}H NMR spectroscopic methods, electronic structure calculations and CD spectroscopy. The biological effects of the dermacozines compromised antitumor, antiparasitic and antioxidative activities [2].
We show the identification of the phenazine gene cluster in *Dermacoccus* sp. MT1.2. A genome library of strain MT1.2 was screened by colony PCR. On cosmid MW_A9 a possible gene cluster was found that contained the essential phenazine core genes and some more genes involved in the modification of the intermediate product phenazine-1,6-dicarboxylic acid. We also show a proposed biosynthesis of dermacozines with respect to the pathway already known from other phenazine producing bacteria.

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MEP023

On the way to unravel a novel biosynthetic pathway for the unique volatile 'sodorifen' of *Serratia odorifera*

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Bacteria are a profound source of secondary metabolites, e.g. antibiotics and toxins (1). Unexpectedly large and diverse is also the spectrum of volatile secondary compounds. *Octamethyl bicycle* (3.2.1) octadiene (`sodorifen´) a volatile secondary metabolite of *Serratia odorifera* 4Rx13 was recently found and structurally elucidated (2). `Sodorifen´ ($C_{16}H_{26}$) is composed of a new and unusual type of carbon skeleton. Each carbon atom of the bicyclic structure is methylated resp. methylenated. As the structure is new to science also the biosynthesis of this compound is still a mystery. A multi strategy approach including physiological experiments, genome, proteome, and metabolome analysis is presently conducted to unravel the biosynthesis and regulation of `sodorifen´. Feeding experiments with different carbon sources, e.g. amino acids, organic acids and sugars, were performed. The carbon compounds, which resulted in highest `sodorifen´ emission, were subsequently used in [^{13}C] isotope feeding experiments and incorporation into `sodorifen´ was analysed by GC/MS and NMR. Beside the results of the feeding experiments we will present the accompanied proteome and genome approaches.

Acknowledgement: We thank our collaborators G. Gottschalk, R. Daniel, A. Thürmer, J. Voss, R. Lehmann (University of Göttingen, D), M. Glocker and S. Mikkat (University of Rostock, D).

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MEP024

New elaiomycins produced by *Streptomyces* strains

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In our search for novel secondary metabolites by HPLC-DAD screening, strains *Streptomyces* sp. BK 190 and *Streptomyces* sp. Tü 6399 were subjected to a closer scrutiny because of interesting peaks in their HPLC profile of a culture filtrate extract. Strain BK 190 was isolated from a hay meadow soil taken from Cockle Park Experimental Farm in Northumberland, UK. Strain Tü 6399 was isolated from a rhizospheric soil collected in a spruce stand located in the Rammert Forest near Tübingen, Germany. Both strains were assigned to the genus *Streptomyces* by their morphological and chemotaxonomic features and by the sequence of the almost complete 16S rRNA gene.
It was shown by Kim *et al*. that strain BK 190 produces two novel alkylhydrazide antibiotics, named elaiomycin B and C, which showed inhibitory activities against *Staphylococcus lentus* DSM 6672 and towards the enzymes acetylcholinesterase and phosphodiesterase [1].
Strain Tü 6399 produced two novel azoxy antibiotics, named elaiomycin D and E, which showed an inhibitory activity against *Bacillus subtilis* DSM 10, *Staphylococcus lentus* DSM 6672, *Xanthomonas campestris* DSM 1706 and a slight activity towards the enzyme phosphodiesterase 4; elaiomycin E showed a slight activity against acetylcholinesterase.
The new compounds are similar in structure to elaiomycin, which was first described by Stevens *et al*. [2] containing a unique aliphatic *α,β*-unsaturated azoxy group. Elaiomycin exhibits an unusual inhibitory activity against *Mycobacterium tuberculosis*.

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MEP025

Regulation of pristinamycin biosynthesis in S. Pristinaespiralis

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The streptogramin antibiotic pristinamycin, produced by Streptomyces pristinaespiralis, is a mixture of two types of chemically unrelated compounds: pristinamycin PI and PII, which are produced in a ratio of 30:70. Pristinamycin PI is a cyclic hexadepsipeptide, belonging to the B-group of streptogramins, while pristinamycin PII has the structure of a polyunsaturated macrolactone of the A-group of streptogramins. Both compounds alone inhibit the protein biosynthesis by binding to the peptidyl transferase domain of the 50S subunit of the ribosome and are bacteriostatic. The A-group prevents the binding of the aminoacyl-tRNA to the 50S subunit of the ribosome. In contrast, the B-group facilitates the release of the peptidyl-tRNA from the ribosome. Together they show a strong synergistic bactericidal activity, which can reach 100 times of the separate components. The pristinamycin biosynthetic gene cluster is characterized. It covers a region of about 210 kb where genes for PI and PII biosynthesis are interspersed. Moreover, the pristinamycin coding region is interrupted by a cryptic secondary metabolite gene cluster which probably encodes for an actinorhodin-like compound. Seven regulatory genes were identified within the 210 kb region:spbR, papR1, papR2, papR3, papR4, papR5 and papR6. SpbR (S.pristinaespiralisbutyrolactone-responsive transcriptional repressor) is a specific receptor protein for γ-butyrolactones and the global regulator of pristinamycin biosynthesis. papR1, papR2 and papR4 encode proteins that are homologous to SARPs which are pathway-specific transcriptional activator proteins, whereas papR3 and papR5 code both for proteins that belong to the family of TetR repressors. papR6 encodes a protein belonging to the class of response regulators. On the basis of RT-PCR, bandshift and mutant analysis, a preliminary model of the regulation mechanism of pristinamycin biosynthesis was established.

Mast YJ, Wohlleben W, Schinko E.Identification and functional characterization of phenylglycine biosynthetic genes involved in pristinamycin biosynthesis in Streptomyces pristinaespiralis.J Biotechnol. 2010 Dec 10
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MEP026

Activation of a silent phenazine biosynthetic gene cluster from *Streptomyces* reveals a novel phenazine conjugate

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The activation of silent biosynthetic gene clusters is a principal challenge for genome mining strategies in drug discovery. In the present study, a phenazine biosynthetic gene cluster was discovered in the Gram-positive bacterium *Streptomyces tendae* Tü1028. This gene cluster remained silent under a multitude of cultivation conditions, both in the genuine producer strain and in a heterologous expression strain. However, introduction of a constitutive promoter upstream of the phenazine biosynthesis genes led to the production of phenazine-1-carboxylic acid (PCA) and of a new derivative thereof, i.e. a conjugate of PCA and L-glutamine. The linkage of PCA to L-glutamine by amide bond formation was catalyzed by enzymes of the heterologous expression host *Streptomyces coelicolor* M512 and may represent a detoxification mechanism. The gene cluster also contained genes for all enzymes of the mevalonate pathway and for an aromatic prenyltransferase, thereby resembling gene clusters for prenylated phenazines. However, purification and biochemical investigation of the prenyltransferase proved that it does not prenylate phenazines but hydroxynaphthalene substrates, showing very similar properties as NphB of naphterpin biosynthesis (Kuzuyma *et al*., Nature 2005; 435: 983-7).

MEP027

Genetical analysis of the biosynthesis and zinc-regulation of [S,S]-EDDS, a biodegradable EDTA alternative produced by Amycolatopsis japonicum

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EDDS (Ethylene-diamine-disuccinic acid) produced by Amycolatopsis japonicum is a suitable biodegradable alternative for the synthetic chelating agent EDTA, which has become the highest concentrated waste compound in surface waters.
EDDS is isomeric with EDTA and has similar properties. But in contrast to EDTA it contains two asymmetric carbon atoms, resulting in the existence of three optical isomers, [S,S]-EDDS, [R,R]-EDDS and [R,S]-EDDS. A. japonicum produces the biodegradable S,S-configuration of EDDS. The biosynthesis of EDDS in A. japonicum is strictly zinc regulated. A zinc concentration of 5 μM represses the production of EDDS at any time of the fermentation [CEBULLA, 1995].
In a hypothetical EDDS biosynthesis pathway oxalacetate and the aproteinogenic aminoacid diaminopropionic acid (DAP) are covalently bonded to form an intermediate which is subsequently processed in several steps to finally form [S,S]-EDDS [CEBULLA, 1995]. DAP is also used as a building block in other secondary metabolites with elucidated biosynthesis pathway like zwittermicin A and staphyloferrin B [ZHAO, 2008; CHEUNG, 2009]. Genetic screening in A. japonicum using the sequence encoding the DAP-synthesizing enzymes resulted in the identification of a gene region encoding putative EDDS-biosynthesis-enzymes.
To confirm their involvement in the EDDS biosynthesis we compared their transcription patterns of A. japonicum cultures grown in zinc-containing (none EDDS production) and zinc-free (EDDS production) media. The putative DAP-biosynthesis genes are only expressed under EDDS production conditions and are strictly repressed only by zinc and no other divalent metal ion.
By directed mutagenesis and heterologous expression we want to evidence the responsibility of these zinc-repressed genes for the EDDS production.

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MEP028

Analysis of the biosynthesis of astins from *Aster tataricus* and cyclochlorotine from *Penicillium islandicum*

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Astins are cyclic pentapeptides isolated from roots of the plant *Aster tataricus*.The root extract shows potent anti-tumour activity in mouse tests (1). However, the amounts of astins that can be isolated from plants are very low and chemical synthesis is accompanied by negative impacts on the environment. Therefore, the project ‚Multi enzyme systems involved in astin biosynthesis and their use in heterologous astin production (MESIAB)‘ aims at enhancing the production of astins using molecular genetic tools. So far, astins A-J are known. Cyclochlorotine, a secondary metabolite with high similarity to astins, has been isolated from the fungus *Penicillium islandicum*. Cyclochlorotine is a hepatotoxic compound causing necrosis, vacuolation of liver cells and development of blood lakes (2). Because of the high similarity of the peptides (3), similar enzymes should be involved in the biosynthetic pathways of astins and cyclochlorotine. Both metabolites contain a dichlorinated pyrrole carboxylic acid derivative which is most likely derived from proline. It is assumed that chlorination occurs on the level of a peptide carrier protein tethered pyrrol carboxylic acid moiety by a flavin-dependent halogenase. The anticarcinogenic activity of astins relies on the cyclic peptide and on the chlorinated proline residue (4,5). So far, neither a flavin-dependent halogenase nor nonribosomal peptide synthethases have been described in plants. Via HPLC-MS from extracts of dry roots of *Aster tataricus* all types of astins could be detected, as well as cyclochlorotine from culture media of *P. islandicum*. For genetic analysis we are in the process of sequencing the genome of *P. islandicum* and constructing cDNA-libraries for *A. tataricus* and *P. islandicum*.

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MEP029

Secondary metabolism and morphogenesis in the penicillin producer *Penicillium chrysogenum* is regulated by the *velvet*-like complex

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The recent discovery of a velvet complex containing several global regulators of secondary metabolism in the model fungus *Aspergillus nidulans* [1,2] raises the question whether similar type complexes direct fungal development and secondary metabolism in genera other than *Aspergillus*. The filamentous fungus *Penicillium chrysogenum* is the main industrial producer of the pharmaceutically relevant beta-lactam antibiotic penicillin. All three biosynthesis genes are found in a single cluster and the expression of these genes is known to be controlled by a complex network of global regulators.
Here we provide a functional analysis of a *velvet*-like complex in a *P. chrysogenum* producer strain that underwent several rounds of UV mutagenesis during a strain improvement program [3,4]. This complex

comprises several structurally conserved velvet-like proteins that have distinct developmental roles, illustrating the functional plasticity of these regulators. We performed extensive phenotypic characterizations of single and double knockout mutants using the codon-optimized FLP/*FRT* recombination system [5]. Data from penicillin bioassays and quantification of conidiospores of these knockout mutants clearly show that all velvet-like proteins are involved in secondary metabolism and other distinct developmental processes. By detailed fluorescence microscopy and protein-protein interaction studies using bimolecular fluorescence complementation, tandem-affinity purification and yeast two-hybrid, we want to extend the analysis of the *velvet*-like complex in *P. chrysogenum*. Our results widen the current picture of regulatory networks controlling both fungal secondary metabolism and morphogenesis, which is significant for the genetic manipulation of fungal metabolism as part of industrial strain improvement programs.

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MEP030

Phenguignardic acid and guignardic acid, phytotoxic secondary metabolites from the grape black rot fungus *Guignardia bidwellii*

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The causal agent of black rot on grapes is the phytopathogenic fungusGuignardia bidwellii. Black rot is one of the most devastating diseases on grapes and since 2002 a serve outbreak of the disease was evident in some German winegrowing regions. The infection was observed in abandoned vineyards primarily, but subsequently an expansion to cultivated vineyards was found. The disease can result in significant crop losses ranging from 5 to 80 % of the total yield.
The infection cycle ofGuignardia bidwelliiis characterized by two phases, a symptomless initial phase followed by a necrotrophic phase. Thus the fungus is classified as a hemibiotrophic pathogen. Phytopathogenic fungi often produce phytotoxins for a successful colonisation of the plant. Such low-molecular compounds are frequently involved in disease symptom formation.
Bioactivity guided isolation led to the identification of phenguignardic acid, a new secondary metabolite from submerged cultures of the grape black rot fungus as phytotoxic agent. The compound is structurally related to guignardic acid, a dioxolanone moiety containing metabolite isolated previously fromGuignardiaspecies. However, in contrast to guignardic acid, which is presumably synthesised via deamination products of valine and phenylalanine, the biochemical precursors for the biosynthesis of the new phytotoxin appears to be exclusively phenylalanine.
Both compounds were characterised in biological assays by using vine leaf segments or intact plants. During fermentation optimisation seven structurally related secondary metabolites were detected and isolated. Four of the seven secondary metabolites were found to be phytotoxic on vine leaf segments.

MEP031

The genetic potential of *Streptomyces collinus* Tü 365 to synthesize secondary metabolites

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Streptomycetes are common producers of secondary metabolites like antibiotics. The strain *Streptomyces collinus* Tü 365 is known to produce the antibiotic Kirromycin (Wolf and Zähner, 1972; Weber et al., 2008). By bioinformatic analysis using the antiSMASH software (a secondary metabolite prediction tool, Medema et al., 2011) 26 additional secondary metabolite gene clusters were identified in the genome of this strain, but their function and their biosynthesis products remain to be elucidated. The genome harbors five clusters for NRPSs, five for terpenes, four for PKSs and four for PKS-NRPS-hybrids. Moreover, there are clusters for three siderophores, a bacteriocin, an ectoin, a melanin and a lantibiotic present. Most of the clusters are not expressed or expressed at very low levels under standard laboratory conditions, but gene expression can be induced under certain conditions.
Here we show transcriptional analyses of some of these gene clusters. First, the strain was cultivated in different growth media to analyze the level of expression of the key genes from selected biosynthetic gene clusters by reverse transcriptase PCR (RT-PCR). RNA samples were taken at different time points from the various liquid cultures. The obtained gene expression data will facilitate the identification of the desired compounds.
In a parallel approach cluster 1, a lantibiotic-like gene cluster, was investigated in heterologous expression studies. Cluster 1 consists of a two-gene transporter, a putative lantibiotic prepeptide and a rare putative class IV lantibiotics cyclase. The prepeptide was expressed in *E. coli* and the prepeptide in combination with the cyclase was expressed in *S. lividans* Tk 23.

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MEP032

Identification of Gene Clusters for Biosynthesis of Bromotyrosine in Metagenomes of the Marine Sponges *Ianthella basta* and *Aplysina cavernicola*

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Marine sponges (*Verongida*) are able to produce a set of bioactive molecules. Among those compounds are bromtyrosines and bromotyrosine derivatives. Bromtyrosines (Bts) are known to have pharmacological relevance. In marine sponges, Bts are typically located within the sponging/chitin based skeleton. They are supposed to protect the chitin skeleton from degradation, through chitinase inhibition. Bts from the species *Ianthella basta* and *Aplysina cavernicola* have already been detected, but were not further investigated so far. From other biosynthetic pathways, for example the biosynthetic gene cluster of the peptide antibiotic balhimycin, it is known, that halogenation of tyrosine residues is catalysed by flavin-dependent halogenases. It should thus be possible to detect the Bt-biosynthesic gene cluster of *I.basta* and *A. cavernicola* by using the degenerated PCR primer pair TyrhalA_for/rev which is specific for flavin-dependent tyrosine halogenases. Sponges are known to be associated to a large amount with bacterial symbionts. Therefore, it seems quite likely that the bromotyrosine producer is rather a bacterial or fungal symbiont than the sponge itself. To define the origin of the detected genes, two different methods for the extraction of metagenomic DNA (eDNA; e = environmental) are used. With the first method, the whole eDNA of sponges is isolated, whereas the second method uses an additional symbiont-enrichment-step prior to eDNA extraction. After detection of the halogenase gene, it should be possible to identify the whole gene cluster by using a DNA library (in form of a fosmid library). Finally, the flavin-dependent halogenases will be characterised with respect to its halogenating activity and substrate specificity.

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MEP033

Purification and cloning of the *O*-Methyltransferase of *Alternaria alternata*

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Black-moulds of the genus Alternaria contaminate many foodstuffs and agricultural products. In addition to the economical damage these fungi can produce harmful secondary metabolites, the Alternaria toxins. Some of these mycotoxins such as alternariol (AOH), alternariolmonomethylether (AME), altenuene (ALT) are polyketides and AOH is produced via the polyketide pathway. AOH is than methylated by the alternariol-o-methyltransferase, transferring a methyl group from SAM to AOH to yield AME. The enzyme was partially purified and characterized, but the sequence is still unknown (1, 2).
The Genome of *Alternaria alternata* was sequenced by the group of Chris Lawrence (3). In the genome 11 putative genes coding for polyketide synthases were identified by Blast-analyses (4). Next to some of these polyketide synthetase genes for methyltransferases were also found. As the genes for secondary metabolite production are usually clustered (5), it is likely,

that the genes for AOH polyketide synthase and AOH-*O*-methyltransferase are in close proximity. Therefore identifying the alternariol-*O*-methyltransferase will also reveal the responsible polyketide synthetase.
Putative methyltransferases were also identified by BLAST-analysis in the genome of the close relative *A. brassicicola* and the sequences were used to clone several SAM dependent methyltransferases of *Alternaria alternata*. Three partial and one total sequence were cloned.
With the active expression of the identified genes being not easy, the alternariol-*O*-methyltransferase of *Alternaria alternata* was also characterized in crude extracts and partially purified. An SAM dependent activity-test was developed to identify the enzyme. The products were analysed by HPLC. With the N-terminal sequence of the enzyme it should be possible to determine the gene.

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MEP034

The friulimicin producer *Actinoplanes friuliensis*

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Friulimicin, a lipopeptide antibiotic produced by the rare actinomycete *Actinoplanes friuliensis*, is active against a broad range of multiresistant gram-positive bacteria such as methicillin-resistant *Enterococcus sp*.and *Staphylococcus aureus* (MRE, MRSA) strains.
The complete biosynthetic gene cluster was characterized by sequence analysis and four different regulatory genes (*regA, regB, regC* and *regD*) were identified within the cluster (Müller *et al*., 2007).
Knockout-mutants missing the regulatory genes *regC/D* showed non-production of friulimicin as well as deficiency in carotenoid pigment synthesis which indicates a pleiotropic mechanism of action of the encoded bacterial two component system.
An *in silico* analysis of the *A. friuliensis* genome revealed the presence of several fatty acid biosynthesis genes outside of the known biosynthetic gene cluster, that might be involved in biosynthesis of the lipid part of the antibiotic. Among others three putative FabH genes (β-Ketoacyl-Acyl Carrier Protein Synthase III) could be identified.
To verify the role of these genes in antibiotic biosynthesis transcription analysis by RT Realtime-PCR as well as gene inactivation experiments are carried out.
Moreover three so far unknown secondary metabolite NRPS- and one PKS-gene cluster as well as genes responsible for carotenoid biosynthesis and flagella formation could be identified and are under further investigation.
To study the formation of spore flagella the growth conditions for sporangia formation and sporulation were determined and analyzed by scanning electron microscopy and RT-Realtime PCR. Additionally different methods for enrichment of spores were tested to improve and facilitate the intergeneric conjugation procedure for *A. friuliensis*.

MEP035

Inhibition of quorum sensing in Gram-Negative bacteria by a staphylococcal compound

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Bacteria use signal molecules to regulate population density in a process of bacterial communication called quorum sensing. This process plays critical roles in regulating various physiological activities, including production of antibiotics, secretion of virulence factors, formation of biofilms, swarming motility, bioluminescence, sporulation as well as symbiosis. Similarly, it is found that various bacteria are able to secrete compounds for inhibiting, inactivating or stimulating quorum sensing signals in other bacteria. In our previous study on coinfection ofStaphylococcusandPseudomonas aeruginosa, we observed thatP. aeruginosacould repress the growth of pathogenic staphylococcalspeciesbut not of nonpathogenic staphylococcalspeciesby respiratory inhibitors [1]. Meanwhile, to our surprise, some strains of the nonpathogenic staphylococcalspeciesexhibit unknown compound X to interrupt the function of quorum sensing-controlled factors in gram-negative bacteria, such as the red prodigiosin pigment inSerratia marcescens, the blue-green pyocyanin inP.aeruginosaand bioluminescence inVibrio harveyi.Physical analysis using XAD-16 resin and dialysis membrane demonstrated that the molecular weight of compound X is below 2 kDa. Moreover, compound X resists alkaline and acid pH, high temperature and proteinase K treatment, which might exclude compound X as a normal peptide. However, the mechanism of compound X expression is still unknown since it is independent of the growth temperature, and oxygen concentration in the medium. In further study, not only purification and identification of the compound X using high-performance liquid chromatography (HPLC) and mass spectrometry (MS) are essential. It also needs to indentify the corresponding genes by transposon mutagenesis and cloning random chromosomal DNA of compound producing staphylococcal stain into a nonproducing strain.In the end, investigation of how compound X disrupts the quorum sensing signaling system in gram-negative bacteria would be an important and interesting issue for new generation of antibiotics.

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MEP036

Effect of gallidermin on biofilm of *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Staphylococcus aureus and *S. epidermidis* are widely involved in minor to severe infection. A major problem is the arising of highly virulent and multiple resistant clones and the manifestation of persistent infections due to biofilm-forming strains. Once a biofilm is formed during infection, particularly implant-associated infections, therapy is extremely difficult due to the antibiotic resistance in a biofilm community. The objective of this study was to investigate the activity of gallidermin with respect to prevent biofilm formation and to kill staphylococci once a biofilm has been formed. For planktonic grown *S. aureus* and *S. epidermidis* the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values of gallidermin was in the order of 4-8 μg/ml. This gallidermin concentrating is also sufficient to prevent biofilm-formation of both species representatives. Also, the viability of 24 h and 5-day staphylococcal biofilm grown cells is significantly decreased after treated with gallidermin. We also investigated the effect of gallidermin on the expression of biofilm-mediating genes such as major autolysin (*atl*) and PIA-synthesizing intercellular adhesin (*ica*). Northern blot analysis revealed that in the presence of gallidermin the corresponding transcripts were significantly decreased. Our finding indicates that gallidermin efficiently prevents biofilm formation in staphylococci and represents a good candidate for treatment for appropriate therapy.

MEP037

the cyanobacterial toxin microcystin binds to proteins *in vivo* and plays an essential role in oxidative stress response in *Microcystis aeruginosa*

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Cyanobacteria produce a variety of secondary metabolites with yet unknown functions. Microcystin is one of the most intensely studied secondary metabolites due to its regular involvement in toxic freshwater cyanobacterial mass developments. Here we describe a new function of microcystin acting on proteins of *Microcystis aeruginosa* PCC 7806, which indicates a putative involvement in physiological processes of the producing organism.
The phenotype of the microcystin deficient Δ*mcy*B mutant shows increased susceptibility towards high light conditions of above 300 μE/ $m^2 \cdot s$ when compared to the microcystin producing wild type. Microcystin covalently binds to *Microcystis* proteins *in vivo* and exposition to high light strongly facilitates the binding. *In vitro*, blocking of free sulfhydryl groups of proteins in Δ*mcyB* mutant extracts disables the binding. Accordingly, microcystin most likely interacts with cysteines of *Microcystis* proteins to form a stable thioether bond. One of the most prominent binding partners of microcystin is the large subunit of the carbon fixing enzyme RubisCO (RbcL). Interestingly, the binding of microcystin to RbcL renders the enzyme less susceptible towards proteolysis by the serine protease subtilisin. Comparative proteomic studies revealed altered accumulation patterns of several Calvin cycle enzymes including RubisCO as a consequence to the loss of microcystin production.
Altogether the findings outlined above strongly suggest an important physiological role of microcystin with regard to modifying the proteome of *Microcystis* and increasing the capability of the cells to handle conditions triggering oxidative stress.

Zilliges Y, Kehr J-C, Meissner S, Ishida K, Mikkat S, et al. (2011) The Cyanobacterial Hepatotoxin Microcystin Binds to Proteins and Increases the Fitness of Microcystis under Oxidative Stress Conditions. PLoS ONE 6(3): e17615. doi:10.1371/journal.pone.0017615

MEP038

A new arylsulfate sulfotransferase involved in liponucleoside antibiotic biosynthesis in streptomycetes

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Sulfotransferases are involved in a variety of physiological processes and typically use 3′-phosphoadenosine 5′-phosphosulfate (PAPS) as the sulfate donor substrate. In contrast, microbial arylsulfate sulfotransferases (ASSTs) are PAPS-independent and utilize arylsulfates as sulfate donors. Yet, their genuine acceptor substrates are unknown. Here, we demonstrate that Cpz4 fromStreptomyces sp. MK730-62F2 is an ASST-type sulfotransferase responsible for the formation of sulfated liponucleoside antibiotics[1]. Gene deletion mutants showed that cpz4 is required for the production of sulfated caprazamycin derivatives.
Cloning, overproduction, and purification of Cpz4 resulted in a 58-kDa soluble protein. The enzyme catalyzed the transfer of a sulfate group from p-nitrophenol sulfate (Km 48.1 μm, kcat 0.14 s−1) and methyl umbelliferone sulfate (Km 34.5 μm, kcat 0.15 s−1) onto phenol (Km 25.9 and 29.7 mm, respectively). The Cpz4 reaction proceeds by a ping pong bi-bi mechanism. Several structural analogs of intermediates of the caprazamycin biosynthetic pathway were synthesized and tested as substrates of Cpz4. Des-N-methyl-acyl-caprazol was converted with highest efficiency 100 times faster than phenol. The fatty acyl side chain and the uridyl moiety seem to be important for substrate recognition by Cpz4. Liponucleosides, partially purified from various mutant strains were readily sulfated by Cpz4 using p-nitrophenol sulfate. No product formation could be observed with PAPS as the donor substrate. Sequence homology of Cpz4 to the previously examined ASSTs is low. However, numerous orthologs are encoded in microbial genomes and represent interesting subjects for future investigations.
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MEP039

Tools for the analysis of metagenomic libraries regarding the production of secondary metabolites with biosurfactant properties

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Microbiological produced compounds with tensidic properties (“biosurfactants”) may be useful alternatives for chemical synthesized compounds.
Secondary metabolites like biosurfactants are synthesized in metabolic pathways with individual reactions catalysed by different enzymes. In bacteria, the genes which encode enzymes involved in the same pathway are often organized in gene clusters, meaning they are all localized in one particular region of the chromosome.
In the case of biosurfactants, known gene cluster sizes are between ca. 3.000 base pairs (bp) and up to 70.000 bp.
Aim of this project is the construction of metagenomic libraries with DNA fragments containing clusters encoding enzymes of surfactant producing pathways. Constructing metagenomic libraries requires transfer of genetic information of certain habitats including non-cultivatable organisms which can exclusively live in those niches into a usable form by isolating the DNA directly from the environment and cloning it into appropriate vectors. By expression of the metagenomic DNA in suitable hosts, products of biosynthetic pathways like biosurfactants encoded by this DNA can be identified. Since already the identification is done in well-established and safe expression strains this method not only makes novel compounds available but also ensures an option for recombinant production in these platform production strains. Promising habitats to find strains producing tensidic molecules are fatty and oily environments like slaughterhouses or tannery. Libraries containing DNA originated in such habitats will be screened for producing compounds with tensidic properties.
At present construction of a novel expression vector for library construction is finished which allows the expression of gene clusters encoded in both directions of an inserted DNA fragment. Enabling a rapid workflow as required for efficient work with the large libraries, we have optimized a recently developed fast screening method for biosurfactant production concerning our purposes.

MEP040

Anti-micobial activity of soil-living *Bacillus* species against human pathogenic and sepsis-related bacteria

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Objectives: Soil living bacteria are known to produce compounds that promote plant growth and confer resistance to plant diseases caused by different pathogens [1, 2]. The rhizosphere can be colonized by biofilmformation by various species, thus anti-microbials ensure also survival advantage against competing commensals. For example, *B. amyloliquefaciensstrain* FZB42 secrets at least 12 known antibiotics, which inhibit growth and destroy biofilms of other microorganisms and that belong to different chemical classes: lipopetides, polyketides, small peptides [3]. The aim of our efforts is to scrren culture supernatants of soli living Gram+ for novel substances with activity against biofilms of multi-drug resistant major bacterial human pathogens involved into catheter- and device associated infections.
Methods: We used supernatants of *B. amyloliquefaciens*(n=5), *B. pumilus* (n=1), *B. licheniformis*(n=1) and *P. polymyxa* (n=3) that were filtered, lyophilized and resuspended in 1/10 volume in sterilized water. Supernatants were used in disc diffusion tests against multi-drug resistant isolates of *E. coli* (n=3), *K. pneumoniae* (n=2), *P. auruginosa*(n=4), *S. aureus* (n=3), *E. faecalis* (n=2) and *P. mirabilis* (n=1) as indicator strains. A more detailed analysis of active compounds was performed using bioautography based on thin layer chromatography.
Results: Supernatants of *P. polymyxa* strainsexhibited strongest anti-microbial activity against Gram+ and Gram- pathogens. *B. amyloliquefaciens* FZB 42 showed also high activities against all indicator strains. *B. pumilus* and *B. licheniformis* inhibited mainly growth of Gram+.
Conclusion: Gram-positives soil living bacteria secrete a wide spectrum of bio-active secondary metabolites, which can inhibit the growth of human pathogens. Further experiments will concentrate on identification of particular substances antimicrobial activity and analyze their anti-biofilm activities.
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MEP041

Identification of a gene participating in the sulphur metabolism in Oenococcus oeni

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Sulphur-containing compounds in wine have a high impact on wine flavour and quality. Recent studies demonstrated that Oenococcus oeni is able to produce, from methionine, different volatile sulphur compounds (VSC) (Pripis-Nicolau et al. 2004), but no specific enzymes have been identified and characterised so far.
In this research work an enzyme that degrades sulphur-containing amino acids was identified, heterologous expressed in Escherichia coli BL21(DE3) and biochemically characterised from two O. oeni strains of oenological origins. The amplified PCR product consisted of 1140 nucleotides encoding a deduced protein of 379 amino acids and was highly conserved among the compared O. oeni strains.
The enzyme has characteristics of a cystathionine-g-lyase (EC4.4.1.1), a pyridoxal-5-phosphate-dependent enzyme catalyzing an a,g-elimination reaction of l-cystathionine to produce l-cysteine, a-ketobutyrate and ammonia. Moreover, it was able to catalyse an a,β-elimination reaction synthesizing homocysteine, pyruvate and ammonia from l-cystathionine. An elimination reaction of l-cysteine and dl-homocysteine was also efficiently catalysed by the enzyme, resulting in the formation of H_2S. Furthermore, the ability to demethiolate methionine into methanethiol, an unfavourable volatile sulphur substance, was shown.
Climate change and specific vinification practices can result in wines with high alcohol concentrations (>13 % (v/v)). It could be demonstrated that ethanol contents up to 15 % (v/v) had no impact on the activity of the purified enzymes. Furthermore, the enzymes were stable at temperatures suitable for the wine production and storage. If l-cystathionine was used as substrate, the enzyme activity was highest at pH 8.0. No activity was observed at a pH below 6.5. In, contrast, l-methionine was degraded at pH 5.5 and 6.
Therefore further work with natural substrates will be necessary to determine its influence on the VSC production in wine (Knoll et al. 2011).

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Pripis-Nicolau, L., Revel, G., Bertrand, A. and Lonvaud-Funel, A. (2004) Methionine catabolism and production of volatile sulphur compounds by Oenococcus oeni. J Appl Microbiol 96 (5): 1176-1184

MEP042

Strategies for the recombinant production of the cyclic depsipeptide valinomycin in *Escherichia coli*

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The natural pool of biologically active nonribosomal peptides (NRPs) from bacteria and fungi is vast but still largely untapped. Reasons are the structural complexity of NRPs that impedes chemical synthesis and the poor cultivability of the majority of source organisms. Since nonribosomal peptide synthetases (NRPSs) assemble NRPs from simple building blocks, the heterologous expression of NRPSs in a robust and easy to manipulate expression host like *Escherichia coli* is a desirable strategy to make pharmaceutically relevant NRPs more accessible (1). However, their large size and complexity make recombinant expression of soluble and active NRPSs in *E. coli* a bottleneck.
Valinomycin is a bioactive cyclodepsipeptide formed by the two NRPSs, Vlm1 (370 kDa) and Vlm2 (284 kDa) in *Streptomyces tsusimaensis* (2). In order to establish a recombinant production system for valinomycin in *E. coli* and further characterize the valinomycin biosynthesis, the two *vlm* genes were isolated from the genomic DNA of *S. tsusimaensis* and introduced into various expression vectors via parallel recombinational cloning. A rational expression screening in 24- and 96-well plates was performed to test the expression constructs and relevant cultivation parameters in parallel. Correct folding and activity of the enzymes were assayed *in vitro* after purification. To provide the necessary posttranslational phosphopantetheinylation of valinomycin synthetase the *sfp* gene from *Bacillus subtilis* was genomically integrated into the target *E. coli* expression strain.
We could show that with a high-throughput screening and optimization approach even the large, initially poorly expressed, heterodimeric valinomycin synthetase could be expressed soluble in *E. coli*. *In vitro* activity studies of the four adenylation domains gave information on substrate specificities and experimentally confirmed the postulated mode of action of the valinomycin biosynthetic assembly line (3). Finally, valinomycin formation was achieved by co-expressing Vlm1 and Vlm2 in an engineered *E. coli* strain with genomically integrated *B. subtilis sfp*. This paves the way to tailor the enzymatic assembly line in order to produce nonnatural valinomycin derivatives.

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MEP043

Genomic mining for novel FADH2-dependent halogenases in marine sponge-associated microbial consortia

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Many marine sponges (Porifera) are known to contain large amounts of phylogenetically diverse microorganisms. Sponges are also known for their large arsenal of natural products many of which are halogenated. In this study, 36 different FADH2-dependent halogenase gene fragments were amplified from various Caribbean and Mediterranean sponges using newly designed degenerate PCR primers. Four unique halogenase-positive fosmid clones, all containing the highly conserved amino acid motif “GxGxxG”, were identified in the microbial metagenome of Aplysina aerophoba. Sequence analysis of one halogenase-bearing fosmid revealed notably two ORFs with high homologies to efflux and multidrug resistance proteins. Single cell genomic analysis allowed for a taxonomic assignment of the halogenase genes to specific symbiotic lineages. Specifically, the halogenase cluster S1 is predicted to be produced by a deltaproteobacterial symbiont and halogenase cluster S2 by a poribacterial sponge symbiont. An additional halogenase gene is possibly produced by an actinobacterial symbiont of marine sponges. The identification of three novel, phylogenetically and possibly also functionally distinct halogenase gene clusters indicates that the microbial consortia of sponges are a valuable resource for novel enzymes involved in halogenation reactions.

MEP044

Heterologous Expression of the Lantibiotic Lichenicidin in *E. coli* and Generation of New Congeners by Introducing Non-Natural Amino Acids

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Lantibiotics are a family of ribosomally synthesized peptide antibiotics, produced by various bacteria. Subsequent to their synthesis lantibiotics are posttranslationally modified. Thereby the thioether-containing amino acids lanthionine (Lan) and methyllanthionine (MeLan) are formed from Ser/Cys and Thr/Cys, respectively [1]. The class I and II lantibiotics exhibit antimicrobial activity against a large number of Gram-positive bacteria, e.g. *Staphylococcus aureus*, including MRSA [2], while class III lantibiotics have no antimicrobial effects and display other remarkable bioactivities, e.g. pain-suppression in mice [3].
The class II-lantibiotic Lichenicidin, produced by the Gram-positive *Bacillus licheniformis* is composed of the two subunits Bliα and Bliβ that are synthetized as an inactive prepropeptide (LicA1, LicA2). The peptide is further modified by LicM1 (for LicA1) and LicM2 (for LicA2), exported by LicT and, in the case of Bliβ, it is cleaved by the protease LicP [4]. We developed a system that enabled us to successfully express the biosynthetic genes of Lichenicidin in the Gram-negative host *Escherichia coli* [5].
In order to generate novel structural diversity, we used this powerful tool for genetic code engineering and incorporation of noncanonical amino acids (ncAA). The possibility to express Lichenicidin variants in *E. coli* provides the opportunity for novel Lantibiotics engineering. Ultimately, this will yield novel lantibiotics with new bioactivities due to dramatically increased structural diversity [6].

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MEP045

Characterization of new type-III lantibiotics

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Lantibiotics are a large group of ribosomally synthesized peptides containing the amino acid lanthionine [1]. They are mainly synthesized by Bacilli, Staphylococci, Lactococci and Actinomycetes, and are classified according to their gene cluster, their biosynthetic pathway and their bioactivity into three major subtypes. From type-III lantibiotics produced by Actinomycetes only four peptides (SapB, SapT and LabA1/A2) have been structurally characterized although homologous gene clusters are abundant in other Actinomycetes [2,3,4,5].
All these gene clusters share a similar architecture with all of the encoded prepropeptides containing a characteristic Ser/Ser/Cys motif, which has previously been suggested to act as a precursor of the lanthionine and labionin ring, respectively [4,5]. We report on the detection, analytics and characterization of new type-III lantibiotics. Remarkably, according to our preliminary findings, the new type III-lantibiotics all contain preferably the amino acid labionin. We assume, that these findings have implications for the structures of other type III lantibiotics, [2,3] and suggest that type-III lantibiotics are more abundant than anticipated previously.

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MPV001

The lipodepsipeptide empedopeptin inhibits cell wall biosynthesis through Ca^{2+}-dependent complex formation with peptidoglycan precursors

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Empedopeptin is a natural lipodepsipeptide antibiotic with potent antibacterial activity against multi-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* and penicillin-resistant *Streptococcus pneumoniae in vitro* and *in animal models* of bacterial infection. Here, we present its so far elusive mechanism of antibacterial action.
Empedopeptin selectively interferes with late stages of cell wall biosynthesis in intact bacterial cells as demonstrated by inhibition of N-acetyl-glucosamine incorporation into polymeric peptidoglycan and the accumulation of the ultimate soluble peptidoglycan precursor UDP-N-acetyl-muramic acid-pentapeptide in the cytoplasm. Using membrane preparations and the complete cascade of purified, recombinant late-stage peptidoglycan biosynthetic enzymes and their respective purified substrates, we show that empedopeptin forms complexes with undecaprenyl pyrophosphate containing peptidoglycan precursors. The primary physiological target of empedopeptin is undecaprenyl-pyrophosphate-N-acetylmuramic acid-pentapeptide-N-acetyl-glucosamine (lipid II), which is readily accessible at the outside of the cell and which forms a complex with the antibiotic in a 1 : 2 molar stoichiometry. Lipid II is bound in a region that involves at least the pyrophosphate group, the first sugar, and the upper parts of stem peptide and undecaprenyl chain. Undecaprenyl pyrophosphate and also teichoic acid precursors are bound with lower affinity and constitute additional targets. Calcium ions are crucial for the antibacterial activity of empedopeptin, as they promote stronger interaction with its targets and with negatively charged phospholipids in the membrane. Based on the high structural similarity of empedopeptin to the tripropeptins and plusbacins, we propose this mechanism of action for the whole compound class.

MPV002

The *Staphylococcus aureus* plasmin-sensitive protein Pls is a glycoprotein

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Question: Until recently, the inability of bacteria to glycosylate proteins has been considered a dogma. Now, it is widely accepted that bacteria can glycosylate proteins. Most bacterial glycoproteins identified to date are virulence factors of pathogenic bacteria, i.e. adhesins and invasins. Methods and Results: To study the impact of protein glycosylation in staphylococci, we analysed lysostaphin lysates of the methicillin-resistant *Staphylococcus aureus* (MRSA) strain 1061 by SDS-PAGE and Periodic Acid Schiff stain that specifically stains glycosylated proteins. We detected two glycosylated surface proteins with molecular masses of ~270 and ~180 kDa, the latter being a degradation product of the 270 kDa protein and identified as *pl*asmin-*s*ensitive protein Pls by mass spectrometry. In a search for potential glycosyltransferases (Gtfs) involved in the glycosylation of Pls, we expressed the *pls* gene that is encoded on SCCmec type I in the SA113 wild-type strain and various Gtf mutants (SA113*gtfAB*, SA113*bgt*, SA113*E3*, SA113*gtfABE3*, SA113*gtfABE3E4*). All strains, but the SA113*gtfAB* mutants produced glycosylated versions of Pls indicating a role for GtfA and/or GtfB in Pls glycosylation. However, the MRSA mutant strain COL*gtfAB* still produced a glycosylated version of Pls suggesting that MRSA genomes carry additional *gtf* genes. Blast searches identified two potential *gtf* genes downstream of *pls*, which we termed *gtfC* and *gtfD*. Expression analysis indicated that both, GtfC and GtfD, are involved in glycosylation of Pls in the MRSA strains COL and 1061. Moreover, the construction and characterization of *pls* subclones revealed that glycosylation occurs at the C-terminal SD repeats of Pls. Pls is known to prevent *S. aureus* adherence to fibrinogen and fibronectin and also its internalization by host cells probably acting by steric hindrance. ELISA adherence and internalization assays indicated that these functions are not due to the glycosylation of Pls. However, we detected a significant impact of Pls glycosylation on its binding to peptidoglycan suggesting a potential function in the proper targeting and/or surface display of Pls. Conclusion: The *S. aureus* plasmin-sensitive protein Pls is a glycoprotein and GtfC/GtfD are Gtfs involved in its glycosylation. Glycosylation of Pls has no impact on its ability to prevent adherence or internalization, but potentially plays a role in its proper targeting and/or surface display. Currently, further analyses are on the way to determine the impact of sugar modifications on *S. aureus* pathogenicity, which may represent promising new targets for therapeutic measures.

MPV003

From target to therapy - Expression and characterization of an anti-staphylococcal antibody

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The Gram-positive bacteriumStaphylococcus aureusis the major cause of nosocomial infections. In particular, diseases caused by methicillin-resistantS. aureus(MRSA) are associated with higher morbidity, mortality and medical costs due to showing resistance to several classes of established antibiotics and their ability to develop resistance mechanisms against new antibiotics rapidly. Therefore, immunological strategies based on therapeutic antibodies have the potential to close the gap for an efficient treatment of MRSA.
The focus of our work is theidentification of surface components of staphylococci with potential as an immunodominat antigen. In this regardthe immunodominant staphylococcal antigen A (IsaA) has been identified as a putative target for immunotherapy due to its expression by all clinical strainsinvivo, and its surface exposure. Preclinical experiments revealed protective properties of a monoclonal mouse anti-IsaA antibody (UK-66)in vitroby phagocytosis assays and in mouse infection models. Therefore, this mouse monoclonal antibody was selected for humanization. The hybridoma clone UK-66 was the basis for the identification of the antigen binding domain against IsaA. The coding sequence was used to construct recombinant scFv and scFvFc fragments towards IsaA and to humanize the murine antigen binding domain. The fragments were characterized in their function and specificity by Western Blot analysis, ELISA-studies, immuno-fluorescence analysis and FACS experiments. The results revealed that all constructed fragments posses a high specificity towards IsaA and the property of the antigen binding fragments to detect IsaA on the cell surface of differentS. aureusstrains. After these studies the whole antibody was constructed and its function was characterized by ELISA-studies, FACS experiments and killing assays.
Based on these results the humanized anti-IsaA antibody has the potential for a successful immunotherapy against MRSA.

MPV004

Antibiotic resistance and pathogenicity of NDM-carrying *Acinetobacter baumannii*

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Question: The gram-negative bacterium *Acinetobacter baumannii* causes severe nosocomial infections. The worldwide spread of multidrug resistant *A. baumannii* is a serious global health threat. Aggravation of antibiotic treatment is mainly caused by OXA-lactamases and NDM (New Delhi metallo-beta-lactamase). In this study, we analyzed the prevalence of the mentioned resistance genes in clinical *A. baumannii* isolates. Furthermore, we examined the role of NDM in an infection model since it is suggested to be part of a genomic pathogenicity island.
Methods: DNA from *A. baumannii* clinical isolates was screened by PCR for the presence of NDM and OXA-lactamases and verified by sequencing. Antibiotic susceptibility testing was done using Vitek2 and E-test method. Pathogenicity of NDM- and non-NDM strains was investigated in time-kill-kinetics using the *Galleria mellonella* (larvae of the Greater Wax Moth) infection model.
Results: *A. baumannii* strains with an extended antibiotic resistance profile were isolated from 57 patients from intensive care units between 2001 and 2011. We discovered 38 imipenem-resistant strains; among those, 19 were positive for OXA-23, 3 for OXA-24 and 9 for OXA-58. We detected four NDM-carrying isolates: one NDM-1 positive strain from 2007 and two from 2011. In addition, we found one NDM isolate with a novel point mutation from 2010 which is now being considered as NDM-2. NDM-carrying *A. baumannii* were resistant to all tested antibiotics except the reserve antibiotics tigecycline and colistin.
Time-kill-kinetics in infection experiments using our newly established *Galleria mellonella* infection model revealed no difference between

pathogenicity of NDM- and non-NDM-carrying strains. However, therapy with imipenem reduced the mortality of larvae infected with non-NDM strains significantly. In contrast, imipenem injection did not lower mortality rates of larvae infected with NDM strains.
Conclusion: The majority of imipenem-resistant strains carried the OXA-23 gene. The NDM-1 gene could already be detected in isolates from 2007, two years before NDM-1 was initially discovered. The first NDM mutant, NDM-2, was discovered in an isolate from 2010. With the use of *Galleria mellonella*, we established an infection model for evaluating pathogenicity and antibiotic treatment.

MPV005

Intimin and invasin export their C-terminus to the bacterial cell surface using an inverse mechanism compared to classical autotransport

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A large group of bacterial surface proteins is represented by the family of autotransporter proteins, which belong to the type V secretion system and are found in almost all Gram-negative bacteria. Autotransporter proteins are often important virulence factors and consist of three functional domains: a N-terminal signal sequence, a C-terminal translocator domain and a passenger domain in between. After synthesis in the cytosol and Sec-mediated transport across the inner membrane into the periplasm, the translocator domain forms a beta-barrel pore in the outer membrane, presumably with the help of the Bam complex. Through this pore, the passenger domain is then translocated to the surface of the cell.
A second, unrelated family of outer membrane proteins that expose passenger domains on the bacterial outer surface are the intimins and invasins, nonfimbrial adhesins from pathogenic bacteria, which specifically interact with host cell surface receptors and mediate bacterial attachment or invasion. They are integrated into the bacterial outer membrane with the amino-terminal region, while the carboxy-terminal region of the polypeptide is exposed on the bacterial outer membrane. Whereas the surface-localized parts of the protein are functionally well described, the topology and insertion of the N-terminal membrane domain and the translocation process have not been described. To investigate the topology and the mechanism of translocation in more detail, we had a closer look at the amino acid sequence of invasin and intimin using SignalP, PsiBLAST and HHAlign. Herefrom we got a prediction of the signal peptide and twelve different β-strands, which might build up the β-barrel within the outer membrane. Out of these predictions, we developed topology models of the membrane anchor of invasin and intimin. By introduction of HA-Tags defining the orientation of the translocator domain within the outer membrane and extensive immunofluorescence studies, we were able to confirm our models. Furthermore, we show that the major periplasmic chaperone involved in invasin biogenesis is SurA and that DegP is responsible for quality control if invasin.
Moreover, we came to believe that intimin (Int) and invasin (Inv), two major pathogenicity factors ofE.coliandYersinia, are monomeric autotransporters, with the remarkable difference that their domain order is reversed.

MPV006

Analysis of the interaction of invasive M1 *Streptococcus pyogenes* with human endothelial cells

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Question: *Streptococcus pyogenes* (GAS) is a human pathogen that causes a variety of diseases ranging from superficial infections to severe invasive diseases like necrotizing fasciitis and streptococcal toxic shock like syndrome. Serotype M1 and M3 GAS are most frequently associated with invasive diseases. The M1 surface protein is known to be a major epithelial cell invasin and causes vascular leakage in an animal model. However, to cause an invasive disease, the pathogen has to reach deeper tissue, after overcoming the endothelial cell barrier. The project focuses on the interaction of invasive serotype M1 *S. pyogenes* with human endothelial cells (EC). The aim is to identify possible pathogen- and host cell-associated factors that mediate barrier crossing to elucidate the underlying signaling cascades in ECs.
Methods and Results: Using an in vitro EC infection model we could show that different M1 GAS clinical isolates are able to invade polarized confluent EC monolayers. After 3 hours of infection streptococci co-localize with the marker protein Lamp-1, indicating streptococcal trafficking into the late endosomal/ lysosomal compartment. To test whether streptococci containing phagosomes also fuse with terminal lysosomes, these were pre- loaded with BSA-gold particles and analysed by transmission electron microscopy (TEM). Microscopic images reveal a close association of streptococci with gold particles, indicating fusion of the streptococci containing vacuole with terminal lysosomes. Using an isogenic M1 knock out mutant we demonstrate that the M1 protein is an essential factor for the invasion process into ECs. Furthermore, the entry of M1 GAS could be significantly reduced using antibodies purified from rabbit M1 antiserum. Infection studies using M1- coated latex beads suggest that the M1 protein is not only essential but also can solely mediate entry into ECs. M1- coated latex beads, just like the M1 wt strain, traffic into the late endosomal/ lysosomal compartment.
Conclusion: Serotype M1 GAS have the potential to invade polarized human ECs. The M1 surface protein is the EC invasin of M1 GAS. Thus, M1 protein is the second streptococcal factor identified to allow access into polarized confluent ECs, one of the strongest cellular barriers in the human body.

MPV007

Lipopolysaccharides of Gram-negative bacteria contribute to the creation of heparin-induced thrombocytopenia-eliciting antibodies by binding and conformationally altering platelet factor 4

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Some Gram-negative bacteria have been reported to contribute to the etiology of blood clotting disorders, e.g. heparin-induced thrombocytopenia (HIT) among others. HIT is an IgG-antibody-mediated adverse drug reaction against complexes of the positively-charged chemokine platelet factor 4 (PF4) and the most frequently used anionic anticoagulant in clinical medicine, heparin. Interestingly, even heparin-naïve patients are able to generate IgG-antibodies specific for PF4/heparin complexes as soon as 4 days after exposure to heparin, presumably because these patients have encountered complexes similar to PF4/heparin before such as PF4 bound to anionic bacterial surfaces during infections. Likely candidates for negatively charged molecules on the Gram-negative surface are proteins as well as lipopolysaccharides (LPS).
In this study pre-treatment of bacteria with proteinases showed that proteins play only a minor role in PF4 recruitment to the bacterial surface. However, the components of bacteria binding PF4 have been pinpointed by showing that PF4 interacts with LPS of the Gram-negative model organisms *Escherichia coli* and *Salmonella typhimurium*. Remarkably, *E. coli* and *S. typhimurium* mutants with successively shortened LPS-backbone displayed increasing PF4 binding capacity. The highest binding was detected in the *E. coli* Δ*waa*C and *E. coli* Δ*waa*A mutants lacking both the O-antigens and parts of the core LPS. As the *E. coli* Δ*waa*A mutant lacks in addition to the O-antigens and the inner core heptoses also the 3-deoxy-D-manno-octulosonic acids (KDO) and as mono-phosphate lipid A showed a decreased binding of PF4, the results suggested that the phosphate groups of lipid A are the actual structures contributing to PF4-binding. Human PF4/heparin antibodies could be affinity-purified from patient sera using PF4-coated wild-type *E. coli* as well as PF4-coated mutants. Thus purified antibodies tested positive in consecutive PF4/heparin ELISA and heparin-induced platelet activation assays indicated the exposition of PF4/heparin-like epitopes on PF4-coated wild-type *E. coli* and Δ*waa* mutants.
Hence, recruitment of PF4 to Gram-negative bacteria via lipid A and its phosphate groups induces epitopes on PF4 that can trigger a humoral immune response specific for a wide variety of bacterial species.

MPV008

Staphylococcus epidermidis and Staphylococcus aureus Quorum Sensing System agr Regulates Formyl Peptide Receptor 2 Ligand Secretion and thereby the Activation of the Innate Immune System

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Highly pathogenic Staphylococcus aureus and the opportunistic pathogen Staphylococcus epidermidis secrete phenol-soluble modulin (PSM) peptides.Virulence of S. epidermidis depends mostly on the PSM peptides, which induce chemotaxis in neutrophils and cytokine induction in peripheral blood mononuclearcells (PBMCs). The regulation of PSM secretion and production occurs through the agr regulator. While

chemotaxis and cytokine induction are crucial for infections, the molecular basis of the recognition by leucocytes has remained un known. Here we demonstrate that the human formyl peptide receptor 2 (FPR2) senses S. epidermidis PSMs at nanomolar concentrations. Specific blocking of FPR2 or the down regulation of the PSM genes in the agr mutant led to severely diminished capacities of neutrophils to detect S. epidermidis PSMs. Moreover, Staphylococci developed the quorum sensing system agr to control their detection via human FPR2. Thus, the innate immune system uses a global mechanism to detect bacterial pathogens. Targeting FPR2 may help to manage severe infections induced by different pathogens.

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MPV009

Salmonella Typhimurium Stimulated Transcriptional Response Aids Intracellular Replication

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Bacterial products are recognized by innate immune receptors leading to inflammatory responses that can both control pathogen spread and result in pathology. Intestinal epithelial cells, which are constantly exposed to bacterial products, prevent signaling through innate immune receptors to avoid pathology. However, enteric pathogens such as *Salmonella* Typhimurium, are able to stimulate intestinal inflammation in order to promote bacterial infection. We found that *S*. Typhimurium can stimulate innate immune responses in cultured epithelial cells by mechanisms that do not involve receptors of the innate immune system. By delivering a set of effector proteins including SopB, SopE and SopE2 through its type III secretion system, the bacterium directly activates Rho-family GTPases that subsequently trigger a profound transcriptional reprogramming of host epithelial cells. These modifications support bacterial replication by modifying the intracellular environment.

MPV010

Recruitment of PI3 kinase to caveolin 1 determines the switch from the extracellular to the disseminating stage of gonococcal infection

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Neisseria gonorrhoeae causes mainly local infections but occasionally invades the blood stream thereby initiating disseminating gonococcal infections (DGI). Gonococcal type 4 pili (T4P) stabilize local infections by mediating microcolony formation and inducing anti-invasive signals. Outer membrane porin $PorB_{IA}$, in contrast, is associated with DGI and facilitates the efficient invasion of gonococci into host cells. $PorB_{IA}$ binds to the scavenger receptor expressed on endothelial cells (SREC1) under low phosphate conditions, as found e.g. in the vascular system. Here we demonstrate that both, T4P-mediated inhibition of invasion and $PorB_{IA}$-triggered invasion utilize lipid rafts and signaling pathways that depend on phosphorylation of caveolin-1 at Tyr_{14} (Cav1-pY14). We identified the p85 regulatory subunit of PI3 kinase (PI3K) and phospholipase C gamma1 (PLCγ1) as new, exclusive and essential interaction partners for Cav1-pY14 in the course of $PorB_{IA}$-induced invasion. Active PI3K induces the uptake of gonococci via a novel invasion pathway involving protein kinase C and Rac1. Thus the SREC-I/$PorB_{IA}$ interaction triggers a novel route of bacterial entry into epithelial cells and offers first mechanistic insight into the switch from local to disseminating gonococcal infection.

MPV011

Systems biology of the pathogenic bacterium *Yersinia pseudotuberculosis*

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The rising problem of antimicrobial resistance combined with the shortage of antibacterial drug discovery [1] will result in a decreasing number of steadily working antibiotics. A promising approach to find new targets and therapeutics is the achievement of a better understanding of the *in vivo* link between pathogenicity and metabolism in the underlying pathogens. One of the relevant microorganisms in this field is *Yersinia pseudotuberculosis*, the causative agent of self-limiting enteritis, diarrhoea, mesenteric lymphadenitis or autoimmune disorders [2]. Concerning the invasion of mammalian cells, *Yersinia* is known to have a complex regulatory network which is controlled by nutritional and environmental conditions [3].

Here, we investigate its metabolism on the level of molecular *in vivo* fluxes, using state of art ^{13}C metabolic flux analysis, that is, a core technology from industrial biotechnology to perform system-wide pathway analysis and subsequent design-based strain optimization [4], so far rarely found in the medical field. As starting point for the comprehensive analysis, a computational model of the metabolism of *Y. pseudotuberculosis* was created on basis of available genomic information and implemented into the flux software platform OpenFlux [5]. Combined with ^{13}C isotope experiments, the model allows to quantify all major pathways from central carbon metabolism including glycolysis, pentose phosphate pathway, TCA cycle, anaplerotic pathways as well as anabolism of extracellular product formation.

Using this novel approach, several mutants of *Y. pseudotuberculosis* lacking specific virulence factors are compared to the wild type to study the influence of the corresponding genes on metabolism. Simultaneously performed transcriptome profiling provides the link to the layers of regulation, superimposing the flux network. In further studies the influence of different antibiotic classes in sub-inhibitory concentrations will be unraveled as well as the effects of temperature, a key parameter during the infection cycle of *Y. pseudotuberculosis*.

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MPV012

Shigella IpaD has a dual role in type III secretion system activation

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Type III secretion systems (T3SS) are protein injection devices used by Gram negative bacteria to manipulate eukaryotic cells. In *Shigella*, the T3SS is assembled when the environmental conditions are appropriate for invasion. However, secretion is only activated after physical contact of the needle tip with the host cell generates an activation signal. The signal is transmitted to the cytoplasm where it triggers secretion. First, translocators are secreted which form a pore in the host cell membrane. Second, effector proteins are translocated into the host cell.

The activation process is controlled by components both at the needle tip and in the cytoplasm: At the needle tip, IpaD provides a scaffold for the translocators IpaB and IpaC. In its absence no needle tip is formed, the T3SS secretes constitutively and is unable to sense host cell contact. In the cytoplasm, MxiC acts as a gate-keeper of the T3SS. In its absence, the secretion of pore-forming proteins is decreased and effector proteins are leaked.

Questions: What is the role of the major needle tip protein IpaD in secretion activation at the needle tip and in the cytoplasm? What is the role of the cytoplasmic gate-keeper protein MxiC in translocator secretion?

Methods: We have performed random and site-directed mutagenesis of *ipaD* and *mxiC*, respectively, and analysed the type III secretion profiles, needle tip composition and host cell interactions of the mutants. We have also used protein copurification to analyse protein complexes.

Results: Random mutagenesis of *ipaD* identified two classes of mutants. Class I mutants are affected in signal transduction from the needle tip while Class II are affected in regulation of ordered secretion induction from the cytoplasm. Site-directed mutagenesis identified a negatively charged patch on the surface of MxiC that might be involved in interaction with IpaD.

Conclusions: Our data confirms and extends our understanding of the involvement of the major needle tip protein in secretion activation and adds a completely novel aspect to the present model for prevention of premature secretion, in the absence of an activation signal, from within the bacterial cytoplasm.

MPV013

Bartonella henselae adhesin BadA negatively regulates effector secretion through the VirB/D4 type IV secretion system

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The gram-negative, zoonotic pathogen *Bartonella henselae* is the aetiologic agent of cat scratch disease, bacillary angiomatosis and peliosis hepatis. In recent years, two essential pathogenicity factors of *B. henselae* have been investigated in detail: the trimeric autotransporter adhesin *Bartonella* adhesin A (BadA) and the VirB/D4 type IV secretion system (T4SS). BadA mediates adherence to endothelial cells, binding to fibronectin and secretion of vascular endothelial growth factor (VEGF) in host cells. The VirB/D4 T4SS leads to the formation of invasomes and translocates *Bartonella* effector proteins (Beps) responsible for a variety of reactions in the host cell. Analysis of these pathogenicity factors was performed in two different strains of *B. henselae*, one expressing exclusively BadA, the other one only the VirB/D4 T4SS. Therefore, it remained unclear whether BadA and VirB/D4 T4SS functionally interact or interfere with each other.
We analyzed the role of BadA and VirB/D4 T4SS when both were expressed in *B. henselae* simultaneously. Expression and function of BadA and VirB/D4 T4SS were analyzed in a variety of clinical *B. henselae* isolates. However, most isolates exclusively either expressed BadA or VirB/D4 T4SS. Overexpression of full length or truncated BadA in the VirB/D4 T4SS expressing strain affected the function of the T4SS depending on the length of BadA. In contrast, BadA dependent fibronectin binding, VEGF secretion and adhesion to endothelial cells were not affected by a functional VirB/D4 T4SS. Furthermore, disruption of *badA* in the BadA expressing strain by a transposon activated the VirB/D4 T4SS. In summary, our results indicate, that BadA does not function as a partner adhesin for the VirB/D4 T4SS and, instead, BadA expression negatively regulates expression of the VirB/D4 T4SS by unknown mechanisms.
B. Franz, L. Yun-Yueh and M. Truttmann contributed equally to this work. Also, V. Kempf and C. Dehio contributed equally.

MPV014

A metaproteomic analysis of a human indwelling urinary catheter biofilm dominated by *Pseudomonas aeruginosa*

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Long-term catheterization of the bladder leads inevitably to bacteriuria, but is mostly asymptomatic. Adaptive response of some bacteria to the catheter environment causes an efficient biofilm formation, which can contain 5x10^9 viable cells per centimeter. Up to now, scientists investigated the microbial biofilm-forming community mainly by culture dependent methods, and only little is known about the functional adaptation of the organisms and never a catheter-biofilm from humans was analyzed in depth. Our aim was to analyze a biofilm from a long-term catheterized patient by a metaproteomic approach (1D-PAGE --> LC-ESI-MS/MS) to link structure and function of the microbial community present in the biofilm. *P.aeruginosa* was found to be the predominant colonizer (160 out of 340 bacterial proteins could be assigned to *P. aeruginosa*), but also other bacteria belonging to the Enterobacteriales and Bacteroidales were present, indicating a multispecies biofilm. The results were confirmed by quantitative 16S-ribosomal DNA sequencing. Abundant proteins are involved in iron and nutrient uptake, in the osmotic- and the oxidative stress response. Catheter-associated urine includes a set of secreted proteins which are mainly involved in iron and nutrient uptake. Additionally, the pathogens were isolated and cultured in artificial urine and in LB medium. The proteome and the secretome of *P.aeruginosa* were investigated to elucidate the bladder specific expression and secretion of proteins. In addition, the metaproteome contains factors of the human immune system, i.e. factors of the complement system and neutrophils were found known to play an important role during host defense, indicating a symptomatic bacteriuria. Our findings help to gain a better understanding of bacterial biofilms on urinary tract catheters and unravel bladder specific adaptations.

MPV015

Metabolic adaptations of Pseudomonas aeruginosa during cystic fibrosis lung infections

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Question: *P. aeruginosa* is a major source of nosocomial infections in immuno-compromised patients and the leading cause of morbidity and mortality in patients with cystic fibrosis (CF). While the genetics of adaptations to the CF lung environment during long-term infection have been widely studied, the physiological and metabolic impact on the bacteria is large unknown.
Methods: We used untargeted metabolic profiling (metabolomics) of cell supernatants (exometabolome analysis) to compare 179 strains, representing a series of mostly clonal lineages from 18 individual CF patients. Isolates were collected over time periods ranging from between four to twenty-four years for the individual patients.
Results: We found evidence of metabolic adaptation to the CF lung environment: in particular, acetate production across all strains was highly significantly negatively associated with length of infection ($P < 0.001$, Spearman rank-order correlation), while uptake of metabolically 'expensive' aromatic amino acids (Trp, Phe, Tyr) was increased. In addition to this parallel evolution, we observed a large degree of variation between the different clonal lineages.
Conclusion: Our study has shown evidence of parallel metabolic adaptation of *P. aeruginosa* to the CF lung during chronic infection. However, isolates do not simply seem to converge on one metabolic 'end-stage phenotype', but rather exhibit an unexpected level of metabolic diversity between patients. Our data highlights the usefulness of metabolomic investigation of complex phenotypic adaptations during infection.

MPV016

Pneumococcal surface protein C: a multifunctional pneumococcal virulence factor and vitronectin-binding protein

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Streptococcus pneumoniae is an asymptomatic colonizer of healthy humans but can also cause severe local infections or even life-threatening diseases. A prerequisite for pneumococci to colonize the upper respiratory airways is their capability to adhere directly to host cells or indirectly by interacting with the extracellular matrix (ECM). Pneumococcal attachment is mediated by bacterial cell wall components and surface-exposed proteins, respectively. The major adhesin of pneumococci is the Pneumococcal surface protein C (PspC) which binds to the secretory component (SC) of the human polymeric Ig receptor and also recruits the complement regulatory protein factor H. We have also shown that host-cell-bound vitronectin (Vn), an adhesive glycoprotein present in plasma and the ECM, is exploited by pneumococci as a molecular bridge facilitating their adherence to and invasion into host cells by inducing proteins of the host signal transduction cascades. Although the interaction of pneumococci with vitronectin was demonstrated comprehensively, the pneumococcal adhesin for vitronectin remains unknown. Here we demonstrate that the multifunctional PspC protein is capable to interact with human vitronectin. Depletion of choline-binding proteins from the surface of pneumococci resulted in decreased Vn-binding as analyzed by flow cytometry. Accordingly, PspC-deficient pneumococci showed a lower capability to recruit Vn. PspC was also expressed on the surface of non-pathogenic *Lactococcus lactis*. Similar to pneumococci, the heterologous *L. lactis* but not the lactococcal control strain interacted with immobilized Vn. Moreover, purified PspC protein derivatives competitively inhibited binding of multimeric Vn to pneumococci as analyzed by flow cytometry. Surface plasmon resonance studies were conducted with vitronectin immobilized on a CM5 biosensor chip and different PspC derivatives as analytes. PspC peptides comprising the N-terminal and helical R-domain of the native protein showed a dose-dependent Vn-binding. Results of a peptide SPOT array indicated that a lysine-rich region as well as the SC-binding domain of PspC is probably involved in binding to Vn. In conclusion, PspC exhibits vitronectin-binding activity, and the binding site has been narrowed down to an alpha-helical region in PspC.

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MPV017

Complex c-di-GMP signaling networks mediate the transition between biofilm formation and virulence properties in *Salmonella enterica* serovar Typhimurium

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Upon *Salmonella enterica* serovar Typhimurium infection of the gut, an early line of defense is the gastrointestinal epithelium which senses the pathogen and intrusion along the epithelial barrier is one of the first events towards disease. Recently, we showed that high intracellular amounts of the secondary messenger c-di-GMP in *S. typhimurium* abolished stimulation of a pro-inflammatory immune response and inhibition of invasion of the gastrointestinal epithelial cell line HT-29 suggesting regulation of transition between biofilm formation and virulence by c-di-GMP in the intestine. Here we show that highly complex c-di-GMP signaling networks consisting of distinct groups of c-di-GMP synthesizing and degrading proteins modulate the virulence phenotypes IL-8 production, invasion and *in vivo* colonization in the streptomycin-treated mouse model implying a spatial and timely modulation of virulence properties in *S. typhimurium* by c-di-GMP signaling. Inhibition of the invasion phenotype by c-di-GMP is associated with inhibition of secretion of the type three secretion system effector protein SipA. Inhibition of the invasion and IL-8 phenotype by c-di-GMP (partially) requires the major biofilm activator CsgD and/or BcsA the synthase for the extracellular matrix component cellulose. Our findings show that c-di-GMP signaling is at least equally important in the regulation of *Salmonella*-host interaction as in the regulation of biofilm formation at ambient temperature.

MPV018

Characterization of bacterial strains isolated from community acquired asymptomatic catheter associated urinary tract infections

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Bacterial colonization of urinary tract catheters is a major cause of nosocomial infections. Most investigations focus on catheter isolates from clinical sources. To analyze community acquired catheter infections of elderly patients seven different bacterial isolates from urinary Foley´s catheters of an urologist practice were identified and characterized with regard to their biofilm formation, urea utilization, DNA degradation and hemolysis activity. For eight antibiotics the minimum inhibitory concentrations were determined. *Proteus mirabilis, Morganella morganii, Pseudomonas aeruginosa, Alcaligenes faecalis, Enterococcus faecalis, Stenotrophomonas maltophilia* and *Myroides odoratimimus* were isolated from the catheters. All isolates formed biofilms with *S. maltophilia* and *E. faecalis* showing the strongest biofilm formation. Urease and DNase activity was detected for almost all species. Interestingly, hemolysis was only found for *P. aeruginosa*, *S. maltophilia* and *M. odoratimimus*. Only gentamicin abolished growth on 6 out of seven isolates while kanamycin, ampicillin, nitrofurantoin, tobramycin and cefixime showed almost no effect. Ciprofloxacin and levofloxacin only inhibited the growth of *P. mirabilis* and *M. morganii*. The *M. odoratimimus* isolate was completely resistant against all tested antibiotics. We conclude that biofilm formation, urease and DNase production in combination with antibiotic resistance are essential determinants of opportunistic pathogens in community acquired urinary tract catheter infections.

MPV019

Global discovery of virulence-associated small RNAs in *Yersinia pseudotuberculosis*

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Yersinia pseudotuberculosis is a food-born enteropathogenic bacterium and closely related to the human pathogen *Y. pestis*. In both pathogens the RNA chaperon Hfq is required for full virulence (1) indicating that small RNAs play a crucial role in *Yersinia* virulence. In fact, we found that in *Y. pseudotuberculosis* the post-transciptional Csr system participates in motility, stress resistance and the regulation of virulence genes, e.g. the global virulence regulator *rovA*. RovA controls the expression of early stage virulence genes, which are important for *Y. pseudotuberculosis* to colonize and penetrate the intestinal tract (2). In this study, we used a deep sequencing approach to identify and characterize further so far unknown sRNAs associated with *Yersinia* virulence.
Sequencing of RNA libraries from *Y. pseudotuberculosis* wildtype and an *hfq* mutant grown either at 25°C to stationary phase (simulating environmental conditions/early infection phase) or at 37°C to exponential phase (late infection phase) lead to the identification of 315 putative sRNA out of which 15 were encoded on the *Yersinia* virulence plasmid pYV. The majority of these newly identified sRNAs were only found in pathogenic yersiniae. According to the 454 data, one out of four of these newly found sRNAs is temperature-regulated and about 40% are Hfq-dependent. Expression of selected candidates was further analysed and their influence on virulence investigated.

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MPV020

Fishing for ancient pathogens: A draft genome of a *Yersinia pestis* strain from the medieval Black Death

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The Black Death is considered to be one of the most devastating pandemics in human history. Between 1347 and 1352 approximately 30% -50% of Europeans died of this pandemic. Until recently the causative agent of this epidemic was discussed highly controversial, several pathogens -*Bacillus anthracis*, *Yersinia pestis* or an unknown *Filovirus*- were taken into account as putative agents. Previous genetic studies were often criticized as possible contaminants of modern DNA or closely related soil bacteria. Novel methodical approaches to prove the authenticity of ancient DNA using characteristic damage patterns enabled us to verify *Yersinia pestis* as at least one of the causative agents of the Black Death. For this study 109 samples from skeletal remains of medieval plague victims buried in the East Smithfield cemetery in London were analyzed.
In the next step 98% of the ancient genome of *Y. pestis* from four of the victims was reconstructed to 30-fold genomic coverage. Phylogenetic analysis revealed that the ancient pathogen is ancestral to most recent plague strains and very close to the root of all genome wide sequenced human pathogenic *Y. pestis* strains. These findings indicate that the plague originated as a human pathogen in the late medieval age and suggests that all previous plague epidemics were caused by an extinct or so far not sequenced branch of *Y. pestis* or a different pathogen. Furthermore the ancestral *Y. pestis* strain is highly similar to modern human pathogenic strains and therefore weakens the argument that genetic differences contributed to the higher mortality in the medieval era. Other factors beside the microbial genetics, e.g. environmental changes, vector dynamics, genetic susceptibility of the host populations or a concurrent disease, should now be taken into account to explain the observed higher virulence of the plague during the Black Death pandemic. Thus, the first genome of an ancient bacterial pathogen offers a novel opportunity to study the evolution of pathogens.

MPV021

The YfiBNR signal transduction mechanism reveals novel targets for the evolution of persistent *Pseudomonas aeruginosa* in cystic fibrosis airways

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The genetic adaptation of pathogens in host tissue plays a key role in the establishment of chronic infections. While whole genome sequencing has opened up the analysis of genetic changes occurring during long-term infections, the identification and characterization of adaptive traits is often obscured by a lack of knowledge of the underlying molecular processes. Our research addresses the role of *Pseudomonas aeruginosa* small colony variant (SCV) morphotypes in long-term infections. In the lungs of cystic fibrosis patients, the appearance of SCVs correlates with a prolonged persistence of infection and poor lung function. Formation of *P. aeruginosa* SCVs is linked to increased levels of the second messenger c-di-GMP. Our previous work identified the YfiBNR system as a key regulator of the SCV phenotype. The effector of this tripartite signaling module is the membrane bound diguanylate cyclase YfiN. Through a combination of genetic and biochemical analyses we first outline the mechanistic principles of YfiN regulation in detail. In particular, we identify a number of activating mutations in all three components of the

Yfi regulatory system. YfiBNR is shown to function via tightly controlled competition between allosteric binding sites on the three Yfi proteins; a novel regulatory mechanism that is apparently widespread among periplasmic signaling systems in bacteria. We then show that during long-term lung infections of CF patients, activating mutations invade the population, driving SCV formationin vivo. The identification of mutational "scars" in the *yfi* genes of clinical isolates suggests that Yfi activity is both under positive and negative selection *in vivo* and that continuous adaptation of the c-di-GMP network contributes to the *in vivo* fitness of *P. aeruginosa* during chronic lung infections. These experiments uncover an important new principle of *in vivo* persistence, and identify the c-di-GMP network as a valid target for novel anti-infectives directed against chronic infections.

MPV022

Methionine sulfoxide reductases defend *Salmonella* Typhimurium from oxidative stress and provide bacterial pathogenesis

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Question: Oxidative stress produced by the host during *Salmonella* infection converts methionine to a mixture of methionine-*S*-sulfoxide (Met-*S*-SO) and methionine-*R*-sulfoxide (Met-*R*-SO) [1]. The methionine sulfoxide reductases MsrA and MsrB are known to protect bacteria by repairing oxidized methionine, the former being specific for the *S*-form and the latter being specific for the *R*-form [1,2]. In this study we characterize MsrA, MsrB and a third methionine sulfoxide reductase, MsrC, in *S*.Typhimurium.
Methods: For this study we generated deletion mutants in *S*. Typhimurium using the one-step inactivation via homologous recombination [3]. Phenotypic analyses of *S*.Typhimurium strains included growth experiments, challenging bacteria with exogenous H_2O_2, infection of activated RAW 264.7 macrophages and competitive infection of Balb/cJ mice with *S*. Typhimurium. For biochemical characterization of MsrA and MsrB the proteins were overexpressed in *E. coli*, purified and examined by NADPH linked reductase activity assay.
Results: Here we show that deletion of *msrA* in *S*. Typhimurium increased susceptibility to exogenous H_2O_2 and reduced bacterial replication inside activated macrophages and in mice. In contrast, an Δ*msrB* mutant showed the wild type phenotype. We constructed Δ*msrB* and Δ*msrC* mutant strains in a methionine auxotrophic background of *S*. Typhimurium. The Δ*msrC* mutant but not the Δ*msrB* mutant failed to utilize free Met-*R*-SO. Recombinant MsrA was active against both free and peptidyl Met-*S*-SO, whereas recombinant MsrB was only weakly active and specific for peptidyl Met-*R*-SO. To dissect the role of MsrC in oxidative stress response we compared an Δ*msrC* single mutant and an ΔmsrBΔ*msrC* double mutant, and found that MsrC affects survival of *S*. Typhimurium following exposure to H_2O_2, growth in macrophages and in combination with MsrB also in mice.
Conclusions: Thus in summary, we showed that mutants of *S*. Typhimurium lacking components of the methionine sulfoxide reductase pathway are attenuated in vitro when exposed to H_2O_2, inside activated macrophages and in mice. Previously, MsrA and MsrB were considered to be the principle enzymes of the msr-system that play a role in oxidative stress response. Here we show that in addition MsrC contributes significantly to thwart the damage caused by oxidative stress in *S*.Typhimurium [4].

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MPV023

RNase Y of *Staphylococcus aureus* does not result in bulk mRNA decay but in activation of virulence genes

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Bacteria are able to cope with environmental changes by rapidly altering mRNA expression. Coordinated RNA decay is also essential to allow quick adjustment of RNA levels. Several RNases are involved in RNA decay, processing and maturation of the different RNA species. Sequence homologues of major *Escherichia coli* enzymes cannot be identified in firmicutes. Recently, an essential endoribonuclease, RNase Y, was identified in *Bacillus subtilis* as a key member of the degradosome and proposed to be important for bulk mRNA turnover. Here we analyzed the role of RNase Y homologue *rny* in the human pathogen *Staphylococcus aureus*. In contrast to *B. subtilis*, *rny* is obviously not essential in *S. aureus* since *rny* deletion mutants could readily be obtained. As a model for RNase Y action, we used the processing of *sae*PQRS operon coding for components of a global virulence regulatory system. The most prominent transcript of this operon was shown to be generated by specific endonucleolytic cleavage of a larger autoregulated transcript. In *rny* mutants the *sae*PQRS processing was no more detectable. To gain insight into the expression of genes affected by RNase Y, gene expression profiling between *rny* mutant and wild type was compared through microarray analysis. As expected for an RNase mutant, the mRNA levels of several genes/operons were significantly increased in the *rny* mutant. Accordingly, the half life of one of these operons was shown to be extended from 1.1 to 12.7 min. However, the half-lifes of other mRNA species, including virulence genes and regulators such as *agr*, were not significantly altered in the *rny* mutant. This suggests that in *S. aureus* RNAse Y does not lead to decay of bulk RNA but rather influence mRNA expression in a tightly controlled regulatory manner. Interestingly, there were many genes down-regulated in the *rny* mutant. Among those genes, which are presumably controlled by RNase Y in an indirect way, we could identify various known to be involved in the pathogenesis of *S. aureus*. The promoter activities of those virulence genes (e.g. *hlg* and *spa*) were indeed severely impaired in the *rny* mutants. RNase Y was moreover required for full virulence in a murine *S. aureus* bacteremia model. In summary, in *S. aureus* RNase Y is essential for coordinated activation of virulence genes but does not lead to bulk RNA decay as shown in *B. subtilis*.

MPV024

sarA negatively regulates *Staphylococcus epidermidis* biofilm formation by modulating expression of 1 MDa extracellular matrix binding protein and autolysis dependent release of eDNA

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Biofilm formation is essential for *Staphylococcus epidermidis* pathogenicity in implant-associated infections. Nonetheless, large proportions of invasive *S. epidermidis* isolates fail to show accumulative biofilm growth in vitro. We here tested the hypothesis that this apparent paradox is related to the existence of superimposed regulatory systems suppressing a multi-cellular biofilm life style. Transposon mutagenesis of clinical significant but biofilm negative *S. epidermidis* 1585 was used to isolate a biofilm positive mutant carrying a Tn*917* insertion in sarA, chief regulator of staphylococcal virulence. Genetic analysis found that inactivation of *sarA* induced biofilm formation via over-expression of giant 1 MDa extracellular matrix binding protein (Embp), serving as an intercellular adhesin. In addition to Embp, augmented extracellular DNA (eDNA) release significantly contributed to biofilm formation in mutant 1585ΔsarA. Increased eDNA amounts indirectly resulted from up-regulation of metalloprotease SepA, leading to boosted processing of major autolysin AtlE, in turn resulting in augmented autolysis and release of chromosomal DNA . Hence, this study identifies *sarA* as a negative regulator of Embp- and eDNA dependent biofilm formation, linking SepA-mediated escape from defensin dermicidine with biofilm related protection from phagocytosis. Our data establish a central role of *sarA* as a regulator ensuring *S. epidermidi*s adaptation to hostile environments.

MPV025

CspA of *Borrelia burgdorferi* is a regulator of the alternative pathway

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The Lyme disease spirochete, *Borrelia burgdorferi*, is transmitted to the human host through the bite of an infected tick. Upon entry into the bloodstream the spirochetes are immediately confronted by the host's innate immune system. The complement system is an integral part of innate immunity and in order to establish a persistent infection in the host, Borreliae have evolved a number of sophisticated means to evade complement-mediated killing.
The outer surface protein CspA of *B. burgdorferi* contributes to complement resistance by binding host complement regulators such as factor H (CFH) and factor H-like protein-1 (FHL-1). Here we demonstrate

that CspA can also negatively regulate activation of the alternative pathway independently of its ability to bind CFH and FHL-1.
At first, various recombinant CspA proteins with single amino acid substitutions have been generated. The mutated proteins were screened for their ability to bind CFH and FHL-1 utilizing ligand affinity blotting and ELISA. CspA with substitutions at position 101 ($CspA_{K101E}$) and position 242 ($CspA_{D242A}$) displayed a reduced binding capacity for CFH. A substitution at position 246 ($CspA_{L246D}$) reduced binding of CFH to marginal levels. By contrast, amino acid substitutions at positions 146 ($CspA_{L146H}$) and 240 ($CspA_{Y240A}$) abrogated binding of CFH to negligible levels. Additionally, proteins $CspA_{L146H}$ and $CspA_{Y240A}$ did not bind significant levels of FHL-1 in a ligand affinity blot and $CspA_{L246D}$ showed only marginal binding of FHL-1.
In order to assess whether the mutated CspA proteins, with their reduced or negligible binding capacity for CFH and/or FHL-1, retained their negative regulatory effect on the alternative pathway of complement, we employed a cell-based hemolytic assay. Strikingly, just like the wild-type CspA, all mutated proteins investigated were able to protect rabbit erythrocytes from complement-mediated lysis in the hemolytic assay.
Taken together, our findings indicate that CspA is not only able to negatively regulate the alternative pathway of complement by usurping host regulator molecules such as CFH or FHL-1, but that it can also regulate the alternative pathway independently of those molecules. The challenge remains, to find out precisely where this second regulatory activity in the alternative pathway is targeted.

MPV026

Zwitterionic cell wall polymers of bacterial pathogens-important modulators of T cell dependent infections

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S.aureus is responsible for serious and life-threatening human infections, such as bacteremia, pneumonia, and endocarditis. However the most prominent *S. aureus* infections are skin and soft-tissue infections (SSTIs). In contrast to other types of infections, the microbial factors involved in the pathogenesis of skin infections provoked by *S. aureus* and the underlying host response mechanisms have yet to be studied in detail. Therefore, a comprehensive understanding of the molecular events taking place during the course of a staphylococcal skin infection remains largely elusive.
Recently, the dogma of adaptive immune system activation was challenged by studies that demonstrated the ability of certain microbial zwitterionic polysaccharides to be processed and presented via the MHC II pathway much like peptide antigens (1). Cell wall teichoic acid (WTA) of *S. aureus* is a zwitterionic polymer, and we demonstrate that purified WTA is able to stimulate CD4+ T-cell proliferation in an MHC II-dependent manner (2). We show in both in vitro and in vivo experiments that the zwitterionic charge of WTA is crucial for this activity. The results of T cell transfer experiments and CD4+ T cell deficient mouse studies clearly demonstrate that T cell activation by WTA in *S. aureus* infected tissue strongly modulates abscess formation. The primary effector cytokine produced by WTA activated T cells in vitro is IFN-γ, which we found to be responsible for promoting the early phases of abscess formation in vivo. The later stages of abscess progression and clearance rely on a Th17 type response, indicated by high IL-17 levels in the abscess tissues at late time points. We currently try to understand the development and interplay of T cell populations after MHCII dependent activation by WTA. In addition we want to correlate structural differences in WTA polymers of different Gram-positive bacteria to their T cell stimulatory potential. So far we could demonstrate that the WTA polymer of the skin colonizing commensal *Staphylococcus epidermidis* is in contrast to the WTA of *S. aureus* not able to activate T cells after MHC II presentation. The spacing of the charge centers is shorter in *S.epidermidis* WTA as compared to *S. aureus* WTA since *S. epidermidis* WTA contains glycerol-phosphate repeating units as opposed to the ribitol-phosphate units of *S. aureus* WTA. Our study is both novel and highly important for understanding the molecular basis of the complex pathology of staphylococcal SSTIs. In addition, it provides unique insight on the role of staphylococcal glycopolymers in bacterial virulence, emphasizing the importance of investigating these surface molecules from a new perspective.

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MPV027

Novel murine infection models provide deep insights into the „Ménage à Trois" of *Campylobacter jejuni*, microbiota and host innate immunity

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Background: Although *Campylobacter jejuni*-infections have a high prevalence worldwide and represent a significant socioeconomic burden, it is still not well understood how *C. jejuni* causes intestinal inflammation. Detailed investigation of *C. jejuni*-mediated intestinal immunopathology is hampered by the lack of appropriate vertebrate models. In particular, mice display colonization resistance against this pathogen.
Methodology/Principal findings: To overcome these limitations we developed a novel *C. jejuni*-infection model using gnotobiotic mice in which the intestinal flora was eradicated by antibiotic treatment. These animals could then be permanently associated with a complete human (hfa) or murine (mfa) microbiota. After peroral infection *C. jejuni* colonized the gastrointestinal tract of gnotobiotic and hfa mice for six weeks whereas mfa mice cleared the pathogen within two days. Strikingly, stable *C. jejuni* colonization was accompanied by a pro-inflammatory immune response indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils and apoptotic cells as well as increased concentrations of TNF-α, IL-6, and MCP-1 in the colon mucosa of hfa mice. Analysis of $MyD88^{-/-}$, $TRIF^{-/-}$, $TLR4^{-/-}$, and $TLR9^{-/-}$ mice revealed that TLR4- and TLR9-signaling was essential for immunopathology following *C. jejuni*-infection. Interestingly, *C. jejuni*-mutant strains deficient in formic acid metabolism and perception induced less intestinal immunopathology compared to the parental strain infection. In summary, the murine gut flora is essential for colonization resistance against *C. jejuni* and can be overcome by reconstitution of gnotobiotic mice with human flora. Detection of *C. jejuni*-LPS and -CpG-DNA by host TLR4 and TLR9, respectively, plays a key role in immunopathology. Finally, the host immune response is tightly coupled to bacterial formic acid metabolism and invasion fitness.
Conclusion/Significance: We conclude that gnotobiotic and "humanized" mice represent excellent novel *C. jejuni*-infection and -inflammation models and provide deep insights into the immunological and molecular interplays between *C. jejuni*, microbiota and innate immunity in human campylobacteriosis.

MPP001

Detoxification of nitric oxide during *Salmonella* pathogenesis

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The detoxification of nitric oxide plays a major role during the pathogenesis of *Salmonella* species. Nitric oxide has bactericidal properties and is generated by inducible nitric oxide synthase inside macrophages, where *Salmonella* is able to reside [1]. *Salmonella* has evolved detoxification mechanisms, which allow survival in such a stressful environment. Previous work has revealed the importance of NorV, NrfA and HmpA [2], but more mechanisms are likely to be involved. Microarray analysis of *Salmonella enterica* serovar Typhimurium (*S.*Typhimurium) highlighted the up-regulation of genes that contain putative tellurite resistance domains. The effect of gene deletions has been investigated using various sensitivity and viability assays ranging from exposure to hydrogen peroxide, effects on growth in the presence of tellurite, effect on growth by nitric oxide under oxic and anoxic conditions to the use of cell culture models.

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MPP002

Discriminative hexaplex PCR strategy for the detection of methicillin resistance and virulence factors in *Staphylococcus aureus*

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In clinical microbiology, phenotypic characterization is laborious and time consuming strategy, remains less discriminative among high virulent to less virulent clinically important strains. Induction of molecular techniques, allow a more accurate and less time consuming way out for the

identification of Staphylococcus aureus along with its virulence capabilities. Here we describe a hexaplex strategy for a rapid detection of methicillin resistance, simultaneously discriminating S. aureus from coagulase-negative staphylococci (CoNS) and occurrence of virulence factors. It targets the nuc (specific for S. aureus), mec A (methicillin resistance determinant), fem A and fem B (S. aureus specific factors essential for methicillin resistance), Luk S/F PV (encodes for Panton Valentine Leukocidin-PVL) and spa (encodes protein A). Validation of this strategy was performed using previously characterized clinical isolates of methicillin susceptible Staphylococcus aureus (MSSA), methicillin resistant Staphylococcus aureus (MRSA) and CoNS from different hospital facilities. Amplification results were consistent and perfectly accurate in accordance to the biochemical and resistance properties of the isolates. This molecular approach renders clinical microbiology a feasible, rapid, simple and reliable technique discriminating MSSA, MRSA and CoNS and provides an early and accurate way of detection, contributing in prevention from widespread dissemination and facilitating antibiotic therapy design.

MPP003

Optimization of PCR strategy for multilocus sequence analysis of *Staphylococcus aureus*

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Methicillin-resistant Staphylococcus aureus (MRSA) has been the most common nosocomial pathogen worldwide. It is generally documented as the most significant due to the burden of diseases it causes and to the evolution and global spread of multidrug-resistant clones. This study describes the optimization of PCR assay for the multilocus sequence typing (MLST) and analysis of housekeeping genes harbored by *Staphylococcus aureus* isolates. Conditions were optimized for a total of seven housekeeping genes which are carbamate kinase (arcC), shikimate dehydrogenase (aroE), glycerol kinase (glpF), guanylate kinase (gmk), phosphate acetyltransferase (pta), triosephosphate isomerase (tpi), acetyl coenzyme A acetyltransferase (yqiL) each of which were ~500bp. A total of 50 human clinical isolates of methicillin-resistant and -sensitive Staphylococcus *aureus* were used to validate the method. This assay offers simple, feasible and specific amplification of multilocus products, which would be more precisely and accurately analyzed by direct sequencing

MPP004

Isothermal Microcalorimetry as a powerful technique for susceptibility testing and investigation of multidrug resistant organisms

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The need for finding and testing new drugs against multiresistent organisms is a great challenge for our modern society. With regard to the high amounts of antibiotics present in daily life the problem of resistant organisms will increase in future. Thus, beside improvements in hygiene especially in hospitals and a conscious usage of antibiotics there is great need for developing new drugs and reliable tests to investigate their impacts.
Since Isothermal Microcalorimetry (IMC) relies on dissipated heat over time it is generally applicable to all sorts of organisms. There is hardly any restriction to the media either; IMC might be used with all body fluids, with solutions, broths or agars, fluid or solid. Hence, it has proved to be a simple, powerful and convenient technique to record the growth and metabolism of bacteria, cell cultures and parasites (Braissant et al. 2009). Furthermore it is quicker than established techniques like proportion method on plates or blood cultures (e.g. Howell et al. 2012, Buess 2007). In addition the method is nondestructive and provides a real time detection of the investigated process instead of snapshots which makes it especially valuable for testing the mechanisms of drug effects. It has been shown that drugs can diminish the growth of bacteria but there are also drugs that just delay the onset of growth (e.g. von Ah et al. 2009). To distinguish these two modes of drug effect is almost impossible with common techniques. Therefore IMC is a powerful method to determine minimal inhibiting concentrations (MIC) of drugs and other toxicological approaches. Actually at standardized conditions IMC may reveal diagnostic capabilities because the heat flow curves are characteristic for most species. In environments with just a few frequently present species like bacteria in a hospital the heat flow curve of a blood sample for example may reveal the target species.
This talk gives a short overview of recent IMC studies in the field of microbiology and drug testing with a focus on human pathogenic organisms. It also provides some technical aspects of the method and gives an outlook in possible applications in the future.

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MPP005

ATP cytotoxicity assay in presence of CyaA and CyaA* preprations

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Introduction:Adenylate cyclase toxin (CyaA) toxin is an important virulence factor ofBordetella pertussis,the causative agent of whooping cough, and a potential component of acellular pertussis vaccine. The adenosine triphosphate (ATP) assay is an alternative assay for measuring cytotoxicity as it determines the number of viable cells in a culture based on quantitation of ATP, by a bioluminescence method, which signals the presence of metabolically-active cells.
Material & Methods: The amount of ATP is directly related to cell numbers. J774.2 (grown in RPMI 1640 medium), RBL-2H3 and sheep bone marrow mast cells (grown in DMEM medium) were treated with different concentrations of CyaA or CyaA* for 2 h at 37°C. The CellTiter-GloÔ reagent was added to each sample and bioluminescence output was compared to that of a negative control (untreated) (0% cytotoxicity) preparation.
Results:According to this method 50% cytotoxicity for J774.2 cells was caused by CyaA around 0.02 mg protein/ml but was not achieved by CyaA* up to 1.25 mg protein/ml. Little cell death (<15%) was detected with CyaA* below 0.02 mg/ml. CyaA was less toxic for RBL-2H3 and mast cells than J774.2 cells.
Conclusions: Based on these data, it could be concluded that ATP cytotoxicity assay more sensitive assay for the measurement of cytotoxicity by CyaA toxins. However, CyaA will deplete intracellular ATP by formation of cAMP and, as this assay measures ATP, this may give a false indication of the extent of cell killing.

MPP006

Detailed structural analysis of the binding mechanism of two component systems

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In bacteria two-component systems (TCS) are the predominant group of regulatory signal transduction systems sensing and converting external stimuli into a regulatory readout. In general TCS are made up of a sensor histidine kinase (HK) that after autophosphorylation transfers the phosphoryl group to its cognate response regulator (RR), which than effects changes in bacterial physiology. Although TCSs have remarkable similarities in sequence and structure, only small crucial differences seems to have a major impact, which not only results in a specific regulatory readout but also prevents unwanted cross-talk between non-cognate signalling systems (1). Structural information on signal transduction proteins are a prerequisite to identify the crucial attributes that guarantee specificity.
Based on the HK/RR co-crystal structure solved by the group of Marina and colleagues (2) a structural homology model of the catalytic cytosolic part of the envelope stress HK CpxA in contact with its cognate RR CpxR was developed. The analysis of this model identified critical amino acids located in the interface of CpxA to CpxR that contribute specificity between HK and RR (1). To confirm the functionality of these residues, we preformed crosslinking (Membrane-SPINE) (3) with different variants of the membrane anchored HK CpxA carrying single and double substitutions to monitor the impact of the identified residues on the protein-protein interaction between HK and RR *in vivo*.
Altogether, the structural insides of CpxA in complex with CpxR will strikingly contribute to a better understanding of these central signal transduction systems.

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MPP007

Comparative genomic analysis of 44 *Clostridium difficile* strains

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*Clostridium difficile*i s the main cause of healthcare associated diarrhea worldwide. The *Clostridium difficile* associated disease ranges from self-limited diarrhea to life-threatening colitis. One approach to classify different *Clostridium difficile* strains is the determination of the ribotype. The ribotype depends on differences in the length of the 16S-23S rRNA intergenic spacer region. Current publications on comparative analysis of *Clostridium difficile* strains focused mainly on strains of one ribotype. In contrast we present here a comparative genome analysis of genome sequences of 44 different *Clostridium difficile* strains, belonging to 22 distinct ribotypes.

To investigate the phylogenetic diversity among the 44 *Clostridium difficile* strains we computed 14 different trees based on the nucleotide sequence of 14 different highly conservative marker genes using the ARB software package. For each tree we unexpectedly found that strains of the same ribotype belong to one node of the trees.

Within our dataset we elucidated differences and similarities in gene content within strains of the same ribotype and within strains of different ribotypes by implementing a bidirectional NCBI BLAST. Using this approach we computed conserved and specific genes for the *Clostridium difficile* genomes. We found that the number of conserved genes depends on the ribotype of the strain. In accordance to the tree analysis there is a strong correlation between strains of the same ribotype.

Knowing similarities and differences on the gene level, the third aspect of our analysis covers the detection of Single Nucleotide Polymorphisms (SNP). Using the software MUMmer for the SNP analysis we clarify which genomic regions are more susceptible to SNPs than others. For example we identified one region that seems to be specific for strains of ribotype 078. Generally we found that the number of SNPs depends on the ribotype of the genome.

Altogether the tree analysis unexpectedly proved so far that strains of the same ribotype are more related to each other.

MPP008

Novel strategies for biofilm disruption from metagenomes

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Staphylococcus- and Pseudomonas species` biofilms on medical devices lead to huge hospital associated problems and are difficult to treat [1,2]. We report on metagenomic screening methods and partial characterization of metagenome clones that either inhibit the development of microbial biofilms or hydrolyze established microbial biofilms. A total of 30.000 fosmid clones of two metagenomic libraries have been analyzed for clones that encode proteins interfering with the inhibition of de novo formation of biofilms and lysis of already established microbial biofilms. Tests were performed using S. epidermidis strain 1457 and P. aeruginosa strain PA028. The screenings have been accomplished via an overlay-assay and in micro titer plates. Altogether 10 fosmid clones were identified that strongly inhibited the formation of P. aeruginosa PA028 and S. epidermidis 1457 biofilm formation. 14 fosmid clones inhibit only S. epidermidis 1457 biofilm formation. Furthermore 3 fosmid clones were identified that disrupt already established S. epidermidis 1457 biofilms. Furthermore all 27 identified fosmid clones have been sequenced via Illumina and the ORFs and proteins involved in biofilm phenotypes are currently in characterization.

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MPP009

Co-regulation of multidrug resistance and pathogenicity in *Erwinia amylovora*

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Erwinia amylovora, a plant pathogenic member of the Enterobacteriaceae, causes fire blight on rosaceous plants, especially pear and apple. Fire blight is one of the most devastating plant diseases caused by bacteria in Germany. Especially apple orchards in South Germany are severely affected by this existence-threatening disease, due to the warmer weather conditions favoring disease development. The commercial implications of this plant disease are aggravated by the limited effectiveness of current control measures. A major pathogenicity factor of *E. amylovora* is multidrug efflux mediated by the RND-type pump AcrAB-TolC. It was previously shown that this efflux system confers resistance to a broad range of structurally unrelated compounds including antibiotics, dyes and plant-derived antimicrobial toxins. Moreover, *acrB*- and *tolC*-deficient mutants showed a dramatically reduced virulence on apple rootstocks. The aim of this project is to explore the cause of the attenuated pathogenicity in mutants of *E. amylovora* lacking a component of the AcrAB-TolC system. In *Salmonella enterica*, a human pathogenic enterobacterium, it was shown that a transcriptional activator was responsible for downregulation of numerous genes encoding proteins involved in pathogenicity in an *acrB*-deficient mutant. We will determine whether such a global regulator, responsible for the co-regulation of pathogenicity and multidrug resistance, exists in *E. amylovora*. Beside AcrAB-TolC, three additional RND-type pumps are present in the annotated genome sequences of *E. amylovora*. To determine the role of these multidrug transporters in antibiotic resistance and virulence of *E. amylovora*, transporter-deficient mutants will be generated and characterized.

MPP010

Sublethal concentration of benzalkonium chloride increases the intracellular proliferation of *Listeria monocytogenes in vitro*

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Question: *Listeria monocytogenes(L.monocytogenes*) is a foodborne pathogen able to persist in the food processing environment for months or even years. Some *L.monocytogenes* strains are more resistant than others to certain sanitizers, like benzalkonium chloride (BAC), and therefore represent a continuous source of recontamination of food products. A recent study indicates that BAC affects the expression of stress proteins and also of proteins related to virulence in *L. monocytogenes* (Kastbjerg et al, 2010).

The aim of our study was to assess the effect of sublethal concentration of BAC on the virulence potential of *L.monocytogenes*.

Methods: Three *L.monocytogenes* strains isolated from cheese smear water - Austria (*Lm1*), cheese - Ireland (*Lm2*), and smoked salmon - Denmark (*Lm3*) and the clinical strain EGDe, all serovar 1/2a, were incubated with or without 1.25mg/l BAC for 30 minutes. Invasion and intracellular proliferation after 4 hours were determined in a cell culture assay using Caco-2, a human colonic carcinoma, HepG2, a human hepatocellular liver carcinoma and THP-1, a human acute monocytic leukemia cell line.

Results: The four *L.monocytogenes* strains vary significantly in invasion and proliferation efficiency, in respect to all three human cell types. EGDe showed the highest ability to invade all cell types, followed by *Lm 3*, whereas for *Lm1* and *Lm 2* a significant lower invasion rate was detected.

Incubation with BAC significantly reduced the invasion rate only of EGDe and *Lm3*, while the invasion efficiency of *Lm1* and *Lm 2* was only slightly but not significantly decreased by incubation with BAC.

Surprisingly, 30 minutes exposure to 1.25mg/l BAC increased significantly the intracellular proliferation for all four strains in all three different human cell types.

Conclusions: These results suggest that through the exposure to ‘stress’ caused by sublethal concentrations of disinfectants *L.monocytogenes* might easier adapt to the intracellular environment of the human cells which leads to a higher intracellular proliferation.

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MPP011

Occurence of culturableVibrio choleraefrom LakeVictoria and two rift valley lakes Albert and George, Uganda

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In Uganda the quality and quantity of clean water are already threatened by poor sanitation, pollution, increasing population pressure and deforestation. Links between climate change impacts, clean water and sanitation and human health are significant in Uganda. An investigation into the occurrence ofVibrio choleraeand correlation with environmental factors was conducted from September 2009 to August 2010 in three lakes. Water samples were collectedmonthly from three shore sampling sites in Lakes Victoria (Gabba), Albert (Butiaba), George (Kayanzi) sites. Various environmental parameters were monitored over this period. Enrichment techniques and standard tests were used to detect the presence ofV. cholerae.Seventy five percent (n= 90) of the samples were positive forV. cholerae. Environmental parameters were found to vary with the abundance ofV. choleraeover the seasons. V. choleraewas more frequentlydetected during the dry than in the wet season. Results reveal

that a unit increase in water temperature resulted into a significant decrease (P <0.05) in the presence ofV. choleraewhile an increase in pH caused a corresponding non significant increase in the presence ofV. cholerae. A unit increase in electrolytic conductivity on the other hand led to a significant increase in the numbers of the organism. Environmental factors partly influence the presence ofV. choleraein Ugandan water bodies. Occurence ofV. choleraewas highest in Lake George compared to the other two lakes. Implications for cholera disease control are also discussed.

MPP012

The genome of *Chlamydia psittaci*: a comparative genome analysis

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Background: *Chlamydia psittaci* is a Gram-negative obligate intracellular bacterium and the pathogenic agent of psittacosis [1]. Although this pathogen's primary hosts are birds [2], human infections can occur with clinical symptoms ranging from mild indisposition to septic multi-organ failure and, ultimately, death [3; 4]. As part of an ongoing effort to investigate the genetic basis of virulence and host specificity in this species we sequenced the full length genome of the *C. psittaci* type strain 6BC [5] and compared it to other intracellular bacteria.
Results: The comparison of the genome of *C. psittaci* to other members of the *Chlamydiaceae* family showed a high similarity in gene content and a high level of synteny. Genome-wide patterns of non-synonymous variation suggest strong purifying selection on large parts of the genome. We identified a number of *psittaci*-specific polymorphic membrane proteins (PMPs) of the G family that may be related to host-pathogen interactions and differences in host-range and/or virulence as compared to closely related *Chlamydiaceae*. Only the G family of the polymorphic outer membrane proteins (PMPs) display a high level of evolutionary change. Another group of proteins essential for chlamydial development within the host cell are type III secretion proteins. In silico prediction of type III secretion proteins suggested several putative proteins of so far unknown function for further analysis.
Conclusions: The genome of *C. psittaci* compared to other members of the *Chlamydiaceae* gives insights into the genome organization, as well as the evolutionary dynamics of the PMP family proteins.

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MPP013

Proteomics of *Legionella*-containing vacuoles from *Dictyostelium* and macrophages

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Legionella pneumophila, the causative agent of a severe pneumonia termed Legionnaires´ disease, replicates intracellularly in environmental and immune phagocytes. Upon internalization of the bacteria into phagocytic cells, a unique membrane-bound replication niche is formed, the "*Legionella*-containing vacuole" (LCV). LCVs communicate with endosomes, secretory vesicles and the ER, but fusion with lysosomes is avoided. Internalization, LCV formation and intracellular replication of *L. pneumophila* are governed by the bacterial Icm/Dot type IV secretion system (T4SS) that translocates more than 250 different "effector" proteins into host cells. LCV formation is a complex and robust process, which can only be understood through a global analysis of pathogen-host factor interactions.
We established a simple and fast method to isolate and purify intact LCVs from the social soil amoeba *Dictyostelium discoideum* [1]. This straight-forward two-step protocol comprises the enrichment of LCVs by immuno-magnetic separation using an antibody against an effector that selectively binds to phosphatidylinositol-4-phosphate on LCVs, followed by density gradient centrifugation. The proteome of intact LCVs was analysed by LC-MS/MS and revealed more than 560 host cell proteins. Recently, we adapted this protocol to isolate intact LCVs from macrophages, allowing comparative proteomic studies of different phagocytic host cells. We found a high overlap among host proteins from LCVs of protozoan or mammalian phagocytes and identified a number of small GTPases and other host proteins that previously have not been detected on LCVs. Currently, we confirm the relevance for LCV formation of novel LCV host cell proteins by fluorescence microscopy, pulldown assays and functional tests.

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MPP014

DivIVA affects secretion of virulence related autolysins in *Listeria monocytogenes*

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DivIVA is a well conserved coiled coil protein present in most Gram positive bacteria and has been implicated in division site selection, biosynthesis of peptidoglycan, and endospore formation. DivIVA proteins bind lipid membranes and characteristically accumulate at curved membrane areas, i.e.the cell poles and the division site, to which they recruit various interaction partners. We have studied the role of this morphogen in the human pathogen *Listeria monocytogenes*and our results suggest a novel mechanism of how DivIVA can contribute to cell division. A Δ*divIVA* mutant displayed a pronounced chaining phenotype which we could attribute to reduced extracellular levels of the autolytic enzymes p60 and MurA. We demonstrate that this is due to a malfunction in secretion of these autolysins and various phenotypic comparisons of the Δ*divIVA* strain with a Δ*secA2* mutant suggest that DivIVA influences the activity of the SecA2 secretion route in *L. monocytogenes*. Deletion of *divIVA* also affected swarming motility, biofilm formation, invasiveness, and cell-to-cell spread in cell culture infection models. Thus, our experiments show that DivIVA is an important factor for various listerial traits that are essential for the pathogenicity of this organism.

MPP015

Characterization of predicted *Mycobacterium avium* subsp. *paratuberculosis* transporter proteins

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Mycobacterium avium subsp. *paratuberculosis* (*MAP*) is an intracellular persisting bacterium and the causative agent of Johne's disease, a chronic inflammatory enteritis in ruminants. Moreover, *MAP* is considered to be involved in the development of Crohn's disease in humans. Comparison of the genomes of *MAP* and the most closely related species *Mycobacterium avium* ssp. *avium* revealed a 38kb *MAP* specific putative pathogenecity island with 23 open reading frames (ORF), clustered in three operons. These genes are assumed to be involved in iron metabolism, as some are predicted to encode for proteins related to siderophore synthesis (*sid*-operon) and ferric transport (*fep*-operon). The main subject of this work is the *mpt*-operon (***M**ycobacterium **p**aratuberculosis* **t**ransporter) containing six ORF. The genes and putative proteins of the *mpt*-operon show high similarities to ATP-binding cassette (ABC)-transporters in *in silico* analysis.
Originally, they were predicted to form a single transporter. At present we revised sequence and topology analyses and developed a new two-transporter-hypothesis. MptABC are expected to form an ABC-exporter and MptDEF to be a member of the recently discovered energy-coupling factor (ECF)-importer class. ECF-importers are a novel class of ABC importers for several vitamins, metals and aminoacids, mainly found in grampositive bacteria. They are composed of two ABC ATPase domains (A/A' units), a conserved transmembrane protein (T unit) and a substrate-specific transmembrane protein (S unit or core transporter), unique in structure. The AT-components constitute the energy providing and the S-component the substrate-specific entity.
In order to support our two-transporter-hypothesis and to elucidate putative substrates of the transporter system(s), we performed RNA sequencing of the whole *MAP* transcriptome, as well as real-time PCR. Moreover, we raised antibodies against the Mpt proteins and constructed plasmids, containing the single proteins, the whole operon and the predicted ABC- and ECF-transporters for overexpression and functional analysis of the proteins in the non-pathogenic *Mycobacterium smegmatis* mc^2 155 as a heterologous system.

MPP016

Regulation and functional characterization of the Arginine Deiminase System (ADS) of pyogenic streptococci

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Pyogenic streptococci comprise a large family of pathogenic bacteria including human specific, animal specific, and zoonotic species, leading to similar clinical patterns and diseases. Its spectrums of diseases range from mild infections of the skin to severe and life-threatening septicaemia, necrotizing fasciitis and toxic shock-like syndrome. Similarities in the patho-physiology between *S. canis*, mainly isolated from dogs and cats, but increasingly recognized as a zoonotic agent, and the well characterized human pathogens *S. pyogenes* and *S. dysgalactiae* sub. *equismilis* suggest common virulence factors in these species. One of these virulence traits is the Arginine Deiminase System (ADS) which is widely distributed among pathogenic streptococci and other prokaryotes. As a secondary metabolic pathway, the ADS catalyses the conversion from arginine to ornithine, thereby producing ATP, CO_2, and ammonia. Besides its role in metabolism, the ADS is also upregulated during infection of the host. Moreover, it is speculated that the ability to raise the external pH due to ammonia formation during arginolysis is responsible for overcoming acidic conditions, e.g. in the phagolysosome.
In pyogenic streptococci the ADS consists of seven genes: *arc*R and *flp*S, coding for putative transcriptional regulators, *arc*A, an arginine deiminase, *arc*B, an ornithine-carbamoyltransferase, *arc*C, a carbamate kinase, *arc*D, an arginine-ornithine antiporter, and *arc*T, a putative *Xaa*/*His*-dipeptidase. We could show in RT-PCR and Western Blot analysis, as well as in enzymatic assays, that the ADS is highly upregulated under nutrient starvation and arginine supplementation. Furthermore, glucose effectively represses the ADS expression underlining its function as a secondary metabolic pathway. Preliminary phenotypic analysis using FACS and electron microscopy revealed that ArcA, ArcB, and ArcC are located on the bacterial surface. This would be a prerequisite for neutralizing environmental acidification. However, its exact contributions to virulence remain elusive.

MPP017

Borrelia bavariensis sp. nov. resist complement-mediated killing independent of binding of complement regulators

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Lyme disease, the most prevalent vector-borne anthropozoonosis in Europe, is caused by spirochetes of the *Borrelia burgdorferi* sensu lato complex.
B. burgdorferi sensu lato differ in their resistance to complement-mediated killing by human serum. It is well-known that complement resistance is correlated with the ability of Borreliae to bind host-derived fluid-phase complement regulators of the alternative pathway, factor H (CFH) and factor H-like protein 1 (FHL-1) via distinct molecules termed complement regulator-acquiring surface proteins or CRASPs.
Here, we investigate *Borrelia bavariensis* sp. nov. formerly described as *Borrelia garinii* OspA serotype 4 for elucidating the molecular mechanism of serum resistance. This genospecies showed a higher pathogenicity to humans and displayed an intermediate serum-resistant phenotype to human serum. Interestingly, none of the *Borrelia bavariensis* strains analyzed were able to acquire complement regulators CFH or FHL-1 and did not produce any CRASPs.
To exclude the possibility that *B. bavariensis* captures complement regulators of the classical pathway to escape the innate immune system, the capacity of several isolates to bind C4b-binding protein (C4BP) or C1-Inhibitor was examined. Since none of the borrelial isolates were able to bind these complement regulators, we investigated two CRASP-1 orthologous proteins, BGA66 and BGA71 displaying an intrinsic complement regulatory activity.
Therefore, a serum-sensitive *B. garinii* strain lacking all CRASPs was transformed with a shuttle vector harboring the entire BGA66 or BGA71 encoding gene under the control of their native promoters. Applying growth inhibition assays, the borrelial transformants G1/pBGA66 and G1/pBGA71 survived in 50% human serum. In addition, both strains also showed a strongly reduced deposition of complement components on their surface when compared to the wild-type strain suggesting that BGA66 and BGA71 exhibit complement regulatory activity.
Taken together, we demonstrate that *B. bavariensis* sp. nov. survives in human serum independently of its ability to bind diverse complement regulators including CFH, FHL-1, CFHR-1, CFHR-2, CFHR-5, C4BP, and C1-Inhibitor. In addition BGA66 and BGA71 were identified as candidates for facilitating serum resistance of *B. bavariensis*.

MPP018

Phosphosignaling of human bronchial epithelial cells in response to bacterial virulence factors

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Background: The gram-positive bacterium *Staphylococcus aureus* is a widespread pathogen that colonizes the human skin and the upper respiratory tract. It can cause community- and hospital-acquired infections, including endocarditis, pneumonia, or even sepsis. On the cellular level, *S. aureus* is able to invade host cells and evades the immune response.
Methods: Here, we have employed stable isotope labeling with amino acids in cell culture (SILAC), enrichment of phosphorylated proteins and high-accuracy quantitative mass spectrometry in order to define the host cell response of human bronchial epithelial cells to virulence factors and during invasion and post-invasion by staphylococci.
Conclusion: Human bronchial epithelial cells represent a first line of defense against invading pathogens. Sensing of bacterial products or direct interaction with the pathogenic aggressor leads to a defined change of signal perception and transduction and eventually results in a reprogrammed cellular activity.

MPP019

The evolution of Zygomycetes as causative agents of emergent diseases

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Zygomycetes, formerly described as class within the fungal kingdom, are polyphyletic, and therefore, split into five distinct subphyla, which are the Entomophthoromycotina, Mucoromycotina, Mortierellomycotina, Kickxellomycotina and Zoopagomycotina [1, 2]. The former two subphyla contain species, which are human pathogenic causing infections with diverse predisposition and etiologies. They encompass ubiquitously distributed saprotrophic soil- or dead plant material-inhabiting fungi of the order Mucorales (subphyl.: Mucoromycotina, formerly classified into the polyphyletic class Zygomycetes). Human pathogenic species inhabit different growth temperature optima ranging from 33 °C to 42 °C, while attenuated species and strains exhibit lower temperature optima. Virulence was tested in an embryonated hen egg model. Single and combined genealogies based on distance, maximum parsimony, maximum likelihood and Bayesian analyses of aligned nucleotide sequences of the nuclear-encoded genes for actin (*act*) and for the 5.8S ribosomal RNA flanked by the internal transcribed spacer (ITS) regions 1 and 2 of a total of 150 species were reconstructed. The phylogenetic reconstructions suggest multiple origins of pathogenicity in certain evolutionary lineages. For example, four distinct families, Cunninghamellaceae, Lichtheimiaceae, Mucoraceae and Syncephalastraceae are involved in disease development within the Mucoromycotina [3-9]. Evolutionary trends are discussed with respect to ecology, physiology and virulence.

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MPP020

Induction of the NF-kb signal transduction pathway in response to *Corynebacterium diphtheriae* infection

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Corynebacterium diphtheriae, the causative agent of diphtheria, has been thoroughly studied with respect to toxin production and pili formation. In contrast, knowledge on host responses to infection by this bacterium is limited. In this study, we analyzed epithelial cells in response to colonization by different *C. diphtheriae* isolates.
An NFk-B reporter cell line was used to monitor the effect of *C. diphtheriae* infection on human cells. Adhesion and gentamicin protection assays revealed strains-specific differences in host pathogen interaction. Strain-specific differences and a correlation of invasion rate with induction of NFk-B were observed in luciferase reporter gene measurements. This was further supported by immune-fluorescence microscopy that showed that translocation of p65, as a hallmark of NFk-B induction, was only observed in association with cell invasion by *C. diphtheriae*.
Our data indicate that the response of epithelial cells to *C. diphtheriae* infection is determined by the internalization of bacteria and that invasion of these cells by *C. diphtheriae* is an active process of these bacteria.

MPP021

The Na^+ -translocating NADH:quinone oxidoreductase (Na^+ -NQR) and its role in the bactericidal effect of silver ions on *Vibrio cholerae*

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The antimicrobial effect of silver ions on a broad range of pathogenic microorganisms and even fungi is well known since ancient times. It is still used today in many applications ranging from purification of waste water to lamination of surgical instruments to control bacterial growth [1]. The fact that there are nearly no negative effects on humans make it a promising alternative to common antibiotics. Yet the mechanism by which Ag^+ ions induce cell death or inhibition of growth is not fully understood. One hypothesis describing the bactericidal action of Ag^+ involves inhibition of bacterial respiration [1]. A possible target molecule for Ag^+ is the Na^+ -pumping NADH-quinone:oxidoreductase (Na^+ -NQR). The Na^+-NQR is the main entry point for electrons into the aerobic respiratory chain of many marine and pathogenic bacteria [2]. It is a membrane-bound enzyme complex composed of six subunits (NqrABCDEF) which contains four flavins, one 2Fe-2S cluster and ubiquinone-8 as cofactors [3]. Its primary function is to build up and maintain a sodium motive force (SMF) across the membrane that is used for motility and metabolic work [2]. An inhibition by silver might therefore result in a breakdown of the SMF and the loss of energy.
Here we show that the Na^+-NQR is a target for Ag^+ ions in *Vibrio cholerae*. Its activity is inhibited by Ag^+ in the nanomolar concentration range both in vivo and in vitro.

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MPP022

The role of *Yersinia enterocolitica* YadA, Invasin and host cell β1 integrins for Yop injection into leukocyte populations *in vitro* and *in vivo*

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During *Yersinia* infection, the bacterial type three secretion system (TTSS) is crucial for evasion of the host's immune response. Prior to injection of *Yersinia* outer proteins (Yops) into the targeted cells via the TTSS, bacteria adhere to the host cells via an interaction of YadA or Invasin (Inv) with β1 integrins [1]. It was shown that β1 integrins are crucial for Yop injection into fibroblasts [2]. Levels of Yop injection into leukocytes can be measured by using a β-lactamase reporter system for detection via flow cytometry [2]. *In vitro* infection of splenic leukocytes revealed that DCs, macrophages, B cells and granulocytes are infected in a similar manner with wildtype, Inv- or YadA-deficient strains. Experiments in a mouse infection model revealed that Invasin plays a minor role and YadA a crucial role for Yop injection by *Yersinia enterocolitica*. To investigate the role of β1 integrins for Yop injection into granulocytes, B cells and T cells we derived β1 integrin depleted splenocytes from conditional knockout mice. Depletion of β1 integrins did not affect Yop injection mediated by YadA but reduced Yop injection triggered by Invasin, indicating that only Invasin triggered Yop injection is strictly β1 integrin dependent.
Taken together, our data provide evidence that during systemic mouse infection YadA but not Inv is essential for Yop injection. In consequence this means that during mouse infection Yop injection into leukocytes can occur also in a β1 integrin independent manner.

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MPP023

Streptococcus pneumoniae activates primary human lung cells and stimulates exocytosis of Weibel palade bodies

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Question: *Streptococcus pneumoniae* (pneumococcus) is a facultative pathogenic commensal colonizing the human nasopharyngeal cavity (1). Pneumococci express the pore-forming cytotoxin pneumolysin as a major virulence factor (2). Invasive pneumococcal infections lead to inflammatory infiltration of leukocytes into lung alveoli and to septic dissemination within the vascular system. The lung microvasculature is covered by pulmonary endothelial cells containing special storage granules. These granules are named Weibel-Palade bodies (WPB) and contain the procoagulant von Willebrand factor (vWF) and IL-8, which are released in response to vascular injuries (3). The main question of this study was focused on characterization of the interaction of pneumococci with primary human endothelial lung cells.
Methods and Results: Microscopic analyses of pneumococcal infection with primary human microvascular endothelial cells (HPMEC) revealed a dose-dependent adherence and internalization of pneumococci. Interestingly, measurement of reactive oxygen species production using carboxylated H2-DCFDA indicated an activation of the cells by pneumococci. Moreover, evaluation of changes in the amount of WPB-containing cells demonstrated a stimulation of WPB exocytosis during a pneumococcal infection. The stimulation of WPB-exocytosis was confirmed by biochemical quantification of vWF and IL-8 secretion. In addition, sublytic amounts of pneumolysin stimulated vWF secretion in addition to direct bacterial adherence. Controls of the cell morphology and evaluation of cytotoxic effects confirmed a non-altered fitness of the endothelial cells during the infection experiments.
Conclusions: The release of vWF was induced after infection with pneumococci from both the apical and the basal cell surfaces, indicating a stimulation of WPB exocytosis during septicemia from inside the vasculature and also following invasive pneumococcal transmigration from the pulmonary tissue into the bloodstream. These results demonstrate that pneumococcal infection activates endothelial cells covering the vasculature of humans and induces the release of pro-inflammatory and pro-coagulative components from WPB.

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MPP024

Msb2 shedding protects *Candida albicans* against antimicrobial peptides

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Msb2 is a sensor protein in the plasma membrane of fungi. In the human fungal pathogen *C. albicans* Msb2 signals via the Cek1 MAP kinase pathway to maintain cell wall integrity and allow filamentous growth. Msb2 doubly epitope-tagged in its large extracellular and small cytoplasmic domain was efficiently cleaved during liquid and surface growth and the
extracellular domain was almost quantitatively released into the growth medium. Msb2 cleavage was independent of proteases Sap9/Sap10 and Kex2. Secreted Msb2 was highly *O*-glycosylated by protein mannosyltransferases including Pmt1 resulting in an apparent molecular

mass of >400 kDa. Deletion analyses revealed that the transmembrane region is required for Msb2 function, while the large N- terminal and the small cytoplasmic region function to downregulate Msb2 signalling or, respectively, allow its induction of by tunicamycin. Purified extracellular Msb2 domain protected fungal and bacterial cells effectively from antimicrobial peptides (AMPs) histatin and LL-37. AMP inactivation as not due to degradation but depended on the quantity and length of the Msb2 glycofragment. *C. albicans msb2 mutants* were supersensitive to LL-37 but not histatin-5 suggesting that secreted rather than cell-associated Msb2 determines AMP protection. Thus, in addition to its sensor function Msb2 has a second activity because shedding of its glycofragment generates AMP quorum resistance.

MPP025

Assembly, stoichiometry and turnover of the *Yersinia* Type III secretion system

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The Type III Secretion System represents one of the most complex prokaryotic protein transport systems. The machinery, also called injectisome, spans both bacterial membranes and the periplasm, and allows the direct, tightly regulated transport of effector proteins from the bacterial cytosol into the host cell.
Around 25 proteins are involved in assembly and function of the injectisome. Even though its overall structure could be visualized, our knowledge about assembly, exact composition and dynamic behavior of the functional injectisome remains limited, especially with respect to the actual export apparatus in the inner membrane and the cytosol.
To answer these questions, we have created fluorescently labeled injectisome components in *Yersinia enterocolitica* and analyzed them *in vivo*, which allowed us to determine the stoichiometry and turnover of different substructures.
Beyond assessing assembly, composition and dynamics of the functional machinery, our approach can yield first insights into the adaptation of the injectisome to changes in the environment.

MPP026

Interaction of *Legionella pneumophila* outer membrane vesicles with host cells and bacteria

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Question: *Legionella pneumophila* is a Gram-negative intracellular pathogen that can cause a severe form of pneumonia. After aerosol formation in man-made water systems, *L. pneumophila* can enter, colonize and destroy the human lung. During infection the pathogen employs sophisticated machineries to deliver proteins to host cells and tissues.
Besides the secretion of individual proteins, *L. pneumophila* sheds vesicles derived from its outer membrane. Outer membrane vesicles (OMVs) are spherical bilayer structures and consist of characteristic outer membrane constituents including outer membrane proteins, phospholipids and LPS (Shevchuket al.,2011) as well as periplasmic components.
The group has described a comprehensive proteome reference map for OMVs of *L. pneumophila* (Galkaet al.,2008). A functional classification of the proteome showed that OMVs contain many virulence factors. Confocal laser scanning microscopy revealed a spatial association between *L. pneumophil*a OMVs and the host cell surface. It remains unclear if this indicates adhesion or fusion events between OMVs and host membranes. The role of *L. pneumophila* OMVs in interbacterial communication is also unknown.
Methods and Results: To address the question if OMV material is incorporated into target cell membranes, human macrophages and different bacteria were co-incubated with OMVs. Samples were taken and analysed at various time points. The presence of the *L. pneumophila* major outer membrane protein (MOMP) could not be detected in any of the target cells by Western blotting.
Conclusion: This finding hints towards a weak interaction with cell surfaces or rapid ingestion and degradation of OMV material. Ongoing studies address the effect of *L. pneumophila* OMVs on human macrophages in regard to metabolic activity and cytoskeleton rearrangements. Interbacterial effects of *L. pneumophila* OMVs are dissected by immunofluorescence microscopy and FACS analysis.

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MPP027

Comprehensive pan-genomics of *Corynebacterium diphtheriae*

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One of the most prominent human pathogens is the Gram-positive bacterium *Corynebacterium diphtheriae*, the causative agent of diphtheria. The species is one of the best investigated bacteria in respect to its toxin and the associated iron homeostasis. In the presence of iron, transcription of the *tox* gene is repressed by the iron dependent regulator DtxR. Nevertheless, little is known about the strain-specific differences leading to the wide variety of symptoms caused in humans by *C. diphtheriae*. In order to understand these crucial differences we sequenced ten strains isolated from patients with classical diphtheria, endocarditis, and pneumonia using 454 technology. In addition, we selected the well-characterized laboratory strain C7β^{tox+}and the most prominent vaccine producer strain PW8. Including the previously published genome sequence of *C. diphtheriae* NCTC1329 we herewith present the comprehensive comparative analysis of thirteen *C. diphtheriae* strains and the first characterization of the pan-genome of this human pathogen. Comparative genomics revealed a core genome consisting of 1611 highly conserved protein-coding regions and approximately 65 singletons on average for every sequenced genome. Moreover, analysis of the prophage region comprising the diphtheria toxin gene *tox* revealed that PW8 has been lysogenized by a second copy of the β-prophage, which encodes an additional *tox* gene. As transcription of the *tox* gene is under control of the iron-dependent regulator DtxR, its putative DNA binding sites were predicted. Comparative studies showed that the DtxR regulon of the sequenced strains exhibits differences due to gene loss, gene duplications and gene acquisition. Moreover, the prediction of pathogenicity islands with the software tool PIPS resulted in the detection of 133 pathogenicity islands distributed throughout the sequenced strains.

MPP028

Comparative study of the invasiveness of Salmonella serotypes Typhimurium and Enteritidis for CaCo-2 cells.

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Introduction: Salmonella serotypes are enteroinvasive pathogens which are responsible mainly for gastroenteritis in humans in industrialized countries. Moreover they are one of the most significant causative agents of food poisoning. Human contamination mainly occurs from poultry products, especially eggs and meat. Salmonella enterica serotype Enteritidis is the most frequently isolated serotype during salmonellosis (33%) [1]. This serotype represents a major problem in chicken rearing because infection is very insidious in these animals and they are usually asymptomatic carriers of Salmonella. Bacteria pass to the eggs transovar and probably also retrocecal and chickens contaminate their fellow birds by spreading Salmonella in the environment via excretion. Meat contamination usually results from carcass contamination at slaughtering [1]. 4% of the S. Typhimurium chromosome (about 200 genes) encode for virulence factors. These factors include, to date, five pathogenicity islands, numerous smaller pathogenicity islets, other virulence factors on the chromosome and at least one virulence plasmid. Invasion induced by S. Typhimurium involves denuding of the microvilli and ruffling of the cell surface. These cell surface rearrangements lead to an uptake of the bacterium in large vacuoles [2].
Method: We compared the invasiveness of S. Typhimurium and S. Enteritidis for CaCo2-cells in a gentamicin protection/invasion assay. Cells were grown in tissue culture trays. Subsequently semiconfluent monolayers were infected with Salmonella to achieve multiplicity of infection (MOI) of 100. Adhesion was allowed to proceed for 3 hours. Serial dilutions were plated onto casein-soja-pepton agar plates for viable bacterial counting. For quantification of intracellular bacteria Caco-2 monolayers were treated with gentamicin to kill extracellular bacteria before proceeding with cell lysis and plating. After incubation epithelial cells were lysed and the number of internalized bacterial cells was enumerated by plating serial lysate dilutions on CASO-agar [3].
Results: This study shows that Salmonella penetrates the intestinal cells after 1 hour of infection. After 3-4 hours bacteria begin to divide. We can show that S. Typhimurium has a higher invasiveness than S. Enteritidis even if the number of bacterial cells of S. Enteritidis at the beginning of infection was higher than the number of S. Typhimurium.

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MPP029

A small RNA represses expression of the chemotaxis receptor TlpB in *Helicobacter pylori*

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The intense study and the sequencing of several genomes of *Helicobacter pylori*, one of the most prevalent human pathogens, have contributed much to understanding of its genomic diversity and virulence mechanisms. However, only a few transcriptional regulators have been described in the small *Helicobacter* genome and almost nothing is known about the role of post-transcriptional regulation of gene expression in this pathogenic Epsilonproteobacterium. Until recently, *Helicobacter* was even regarded as a bacterium without riboregulation [1]. However, our recent differential RNA-seq approach based on high-throughput sequencing of cDNA led to the discovery of ~60 small RNA (sRNA) candidates in *H. pylori* strain 26695 including potential regulators of *cis*- and *trans*-encoded target mRNAs [2]. Here we present the functional characterization of one very abundant sRNA, HPnc5490, which is highly conserved in diverse *Helicobacter* strains. Expression profiling on Northern blots revealed induction of this sRNA under acid stress and accumulation in stationary growth phase. Furthermore, bioinformatics-based target predictions indicated that HPnc5490 could directly bind to a G-repeat far upstream in the 5' UTR of *tlpB* mRNA, which encodes for one of the four chemotaxis receptors of *H. pylori* and is assumed to play a role in pH taxis, quorum-sensing as well as in the inflammatory response upon infection in mice [3, 4, 5]. Studying transcriptome as well as proteome changes upon deletion of HPnc5490 revealed down-regulation of *tlpB* on the mRNA as well as protein level. In addition, complementation of HPnc5490 in the unrelated *rdxA* locus restores repression of the TlpB protein. Moreover, we have constructed several sRNA mutants to validate the interaction site between *tlpB* and HPnc5490 *in vivo*. Initial *in vitro* structure probing and toeprinting experiments suggest that down-regulation of *tlpB* via HPnc5490 is rather based on structural rearrangements, transcript destabilization or transcription attenuation than on the translational inhibition by masking the ribosome binding site. Overall, our results confirm *tlpB* mRNA as a first *trans*-encoded target of HPnc5490 sRNA and indicate that this sRNAs could have a role in regulation of chemotaxis in *H. pylori*.

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MPP030

Characterization of putative virulence factors in *Clavibacter michiganensis* subsp. *michiganensis*.

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Clavibacter michiganensis subsp. *michiganensis* (Cmm) enters tomato plants, multiplies in the xylem sap, and subsequently triggers disease symptoms. Due to the fact that the bacterium causes economic losses of agriculturally important crops, it is one of the quarantine organisms in the European Community. When an infection occurs, virulence factors are of great importance. However, very little is known about these proteins. The two natural plasmids of the wild-type strain Cmm382 carry the genes for the virulence factors CelA, which is a ß-1,4-endocellulase, and Pat-1, which is a putative serine protease. Within the 26 other putative serine protease genes of Cmm, four are located on the natural plasmids.
Here, we analyzed the possible importance of these four genes in pathogenicity with fluorescence measurement, insertion mutagenesis, qPCR, and microarray experiments. Furthermore, we established a xylem surrogate medium to carry out infection experimentsin vitroand analyzed the behavior of Cmm gene expressionin this medium. Additionally, we were interested in proteome analysis and carried out mass spectrometry.
In fluorescence and microarray experiments, we saw an increase in the expression of the five serine proteases and other putative virulence factors in the xylem surrogate medium compared with minimal medium. For the proteome analysis, we carried out the first steps to analyze the surface, extracellular, and cytoplasmic proteins after growing in minimal medium compared with the xylem surrogate medium.
With this new medium as well as the different methods, unknown virulence factors can be identified.

MPP031

Serotype- and host-specific colonization of *Yersinia enterocolitica*

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The food-borne enteropathogen *Yersinia enterocolitica* is responsible for up to 6 000 - 7 000 cases of gastrointestinal diseases in Germany per year. Most infections in Europe are caused by the virulent *Y. enterocolitica* serotypes O:3 and O:9. Interestingly, almost all studies on *Y. enterocolitica* have been done on strains of the serogroup O:8.
Enteropathogenic Yersiniae are able to colonize different host organisms which leads to several symptoms or severities of disease. While humans develop gut-associated as well as autoimmune diseases, pigs remain clinically healthy although the porcine intestinal tract can be efficiently colonized by *Y. enterocolitica*. Slaughtered pigs are known to be the most important reservoir of virulent enteropathogenic Yersiniae. Serotype analysis of the isolated strains showed that O:3 is clearly the most prevalent one in pigs.
In this study different *Y. enterocolitica* isolates were analysed regarding protein levels of virulence factors as well as adhesion, invasion and survival properties on murine, porcine and human epithelial and macrophage cell lines. We observed a significant serotype specificity of the bacterial isolates but no host-specific interactions with the different cell lines.
Since different reactions in the host organisms emerge upon *Yersinia* infections, it seemed necessary to analyse *Y. enterocolitica* infections not only in the well established mouse model but also in pigs, which represent the most important reservoir for Yersiniae. To study the dissemination of *Y. enterocolitica* in pigs, we established a minipig colonisation model. 6-8 week old minipigs were infected with *Y. enterocolitica* wildtype strains of different serotypes and the bacterial burden of different organs was determined. We could show that *Y. enterocolitica* O:3 efficiently colonizes the porcine intestinal tract and is better adapted to pigs than other serotypes. In further experiments we focused on the identification and characterization of virulence determinants in *Y. enterocolitica* serotype O:3 that contribute to the better adaptation.

MPP032

Investigation of bacterial growth and expression patterns ofhexRofPseudomonas syringaeharbouring multiple HexR binding sites

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Pseudomonas syringae pv. glycineaPG4180, the causative agent of bacterial blight of soy-bean plants, possesses several virulence factors, one of them being the synthesis of exopolysaccharides. One of them, levan, is a polymer of fructose, which is synthesized from sucrose by two highly similar enzymes termed levansucrases(Lsc). It was hypothesized that transcription of lscis controlled by the hexose metabolism repressor, HexR. HexR controls genes encoding for the Entner-Doudoroff pathway (EDP), the major glucose utilization route in Pseudomonasspecies. Interestingly, a hexRknock-out mutant of P. syringaewas unable to grow on media containing glucose and sucrose. Thus, a new growth medium was formulated containing glutamate instead of glucose and ammonium chloride as the sole carbon and nitrogen source. DNA affinity chromatography and MALDI-TOF analysis demonstrated binding of HexR to the upstream sequence of lscBat 28°C. The effects of multiple HexR binding sites on growth and hexRexpression in PG4180 transformants carrying the upstream sequences of central glucose metabolism genes (intergenic region between eddand gap-1) and the upstream sequence of lscB, respectively, were investigated in liquid media containing either glucose, sucrose, or glutamate as sole carbon source. In contrast to all other transformants, PG4180 harbouring plasmid 8n-edd-gap showed a significant reduction in growth irrespective of the carbon source. This suggested that there was no particular influence of the carbon source on bacterial growth in presence of multiple copies of HexR binding sites. The hexR expression analysis of the transformants carrying 8n-edd-gap and 8n-lscB in glucose- or sucrose-supplemented media revealed that all transformants showed a higher level of hexRexpression at an OD_{600} of 0.5, which decreased with increasing growth. The progressive growth-dependent decrease ofhexR expression suggested a more important role of HexR during the lag and early logarithmic phases of growth. Interestingly, the highest expression ofhexR was observed in the transformant harbouring plasmid 8n-lscB prompting the speculation that this upstream sequence indeed impacted the expression ofhexR.

MPP033

Phosphorylation in *Staphylococcus aureus*, the role of PknB and Stp

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Posttranslational modification of proteins increases there functional diversity. Very important for the function of a variety of enzymes is thereby the switch between an active or inactive status by reversible phosphorylation. In signal transduction pathways these phosphorylation and dephosphorylation events are a key mechanism to response to intra- and intercellular signal.
Although prokaryotes were thought to use predominantly two component systems for signal transduction, genes encoding serine/threonine, tyrosine kinases and phosphatases have been identified in a wide variety of microorganisms. The aim of our work is the analysis of the function and role of the serine/threonine kinase PknB and its corresponding phosphatase Stp in the metabolism and virulence of the major pathogenStaphylococcus aureus. Therefore, we investigated the transcriptomic profile of theS. aureuswild type strain NewmanHG [1] and its isogenic deletion mutants ΔpknB, Δstpand Δstp/pknB. By this approach we identified an influence ofpknBandstpon the expression of major virulence regulators as well as on central metabolic pathways. Furthermore, by using a proteomic approach substrates and interaction partners of this signaling system could be analyzed. Additionally, ΔpknBshowed a higher virulence potential in an in vivo murine infection model whereas Δstpwas significantly attenuated. Our expression and proteomic data together with the in vivo infection results strongly suggest an important role the signal transduction modul PknB and Stp in the metabolism and virulence ofS. aureus.
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MPP034

Host cell adhesion and immune evasion of zoonotic and non-zoonotic MRSA

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During recent years livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), mostly belonging to the clonal complex (CC) 398, have been recognized as a source for human infections with a potential for a major healthcare challenge. So far the pathogenic mechanism of endemic LA-MRSA and of their methicllin-susceptible counterparts (MSSA) for transmission across species barriers, colonization, and disease formation are mostly unknown. Crucial steps towards disease formation include bacterial adhesion to host cell matrix components, invasion of cells and tissues, and evasion from the host immune response. We are addressing these processes by analyzing the ability of epidemiologically relevant zoonotic and non zoonotic MRSA and MSSA to adhere to human and animal cells and cell matrix components that are known to be important for bacterial adhesion. In a second approach, the uptake of zoonotic and non zoonotic MRSA and MSSA by human and porcine blood phagocytes is investigated.
First results indicate a strain-specific ability to adhere to human keratinocytes, different types of human/bovine collagen and immobilized human/bovine plasma fibronectin. The latter reveals a host dependency of some animal isolates. Overall, zoonotic MRSA CC398 isolates displayed a more heterogeneous fibronectin-binding than human hospital-acquired MRSA isolates. In whole blood phagocytosis assays, we observed significant differences in the bacterial uptake by porcine and human blood granulocytes, but no such differences with regard to the origin of the isolates.
Taken together, our data suggest that MRSA derived from animals cannot be easily distinguished from those derived from humans by the adhesive and immune evasive properties investigated.

MPP035

An FNR-Homologue in the aerobic phytopathgenic bacterium *Xanthomonas campestris* pv.*vesicatoria* controls expression of the operon encoding a high-affinity Cytochrome d Oxidase

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The plant pathogen *Xanthomonas campestris* pv.*vesicatoria* (*Xcv*)is an obligate aerobic oxidase-negative γ- proteobacterium that causes bacterial spot disease on pepper and tomato plants. It grows in the intercellular space between plant cells where it must overcome iron- and oxygen-limitation, as well as cope with reactive oxygen species (ROS) and nitric oxide, which potentially form part of the host defense mechanism. Analysis of the genome of *Xcv* revealed a gene (*xcv1871*) encoding an FNR-like transcription factor, which we refer to as FLP (FNR-like protein). In *E. coli* FNR is an oxygen-responsive transcription regulator switching on gene expression under oxygen-limiting or anaerobic growth conditions. FLP from *Xcv* and *E. coli* FNR share 50% amino acid sequence similarity and FLP could partially complement an *E. coli fnr* mutant. Notably the N-terminal Cys residues required for [Fe-S] cluster coordination and the C-terminal E-SR motif, which in FNR recognises the sequence TTGAT - N4 - ATCAA, are conserved, suggesting that FLP is also an oxygen-sensitive transcriptional regulator in this obligate aerobe. The putative FLP-recognition sequence is conserved in front of the *cydAB* operon encoding a high-affinity cytochrome d oxidase. Growth studies under oxygen-limiting conditions demonstrated that *Xcv* can not grow in the absence of oxygen; however, RT-PCR analyses revealed that transcription of the *cydAB* operon was increased when oxygen levels in the growth medium were low. Deletion of the *flp* gene in *Xcv* prevented transcription of the *cydAB* operon and led to a reduced growth phenotype of the mutant in pepper plants. Introduction of the *flp* gene on a plasmid restored both *cydAB* expression and growth *in planta*. Taken together, our studies suggest that FLP might be required to allow optimal growth of *Xcv* under oxygen-limiting conditions during infection of the host plant.

MPP036

Icm/Dot-dependent modulation of phagocyte migration by *Legionella pneumophila*

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Legionella pneumophila, the causative agent of Legionnaires' disease, infects and replicates in macrophages and in free-living amoebae such as *Acanthamoeba castellanii* or *Dictyostelium discoideum* [1]. Following internalization by a host cell, *L. pneumophila* forms a "*Legionella*-containing vacuole" (LCV), which avoids fusion with lysosomes, communicates with the endoplasmic reticulum and allows intracellular replication. LCV formation is governed by the bacterial Icm/Dot type IV secretion system (T4SS) through the secretion of a myriad of “effector proteins”. Some of these effectors target host phosphoinositide lipids or small GTPases and thus subvert eukaryotic vesicle trafficking and signal transduction pathways. Here we investigate the impact of *L. pneumophila* infection on chemotaxis and motility of mammalian and protozoan phagocytes. To this end, an under-agarose cell migration assay was established to monitor the migration behavior of RAW264.7 murine macrophages and *D. discoideum* amoebae towards tumor necrosis factor (TNF) α or folate, respectively. The motility of phagocytes infected with wild-type *L. pneumophila* was severely reduced compared to macrophages or *D. discoideum* infected with *L. pneumophila* icm/dot mutant strains, which cannot grow intracellularly. Our results indicate that *L. pneumophila* impairs phagocyte migration in an Icm/Dot-dependent manner and suggests that some *L. pneumophila* effector proteins interfere with host signaling pathways that are involved in chemotaxis and cell migration. Currently, we characterize in detail the interplay between bacterial and host factors participating in phagocyte motility.
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MPP037

Crystal structure and regulation mechanisms of the Adenylyl Cyclase CyaB from the human pathogen *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a major cause of nosocomial infections, and treatment of *P. aeruginosa* infections is hindered by the bacterium’s high antibiotic resistance. The regulatory network controlling *P. aeruginosa*

virulence provides novel targets for drug development. CyaB is a virulence regulating sensor protein belonging to adenylyl cyclase (AC) Class III, a protein family forming the second messenger cyclic adenosine 3',5'-monophosphate (cAMP) through conserved catalytic domains, which are regulated by a diverse set of fused regulatory domains. CyaB is further regulated by its N-terminal MASE2 domain, which also acts as cell membrane anchor [1].
We describe here the biochemical and structural characterization of CyaB, and its inhibition by small molecules. The tertiary structure of CyaB shows the same folding pattern, as all previously described class III ACs. CyaB indicates subtle differences in active site and inhibitor binding sites by using the AC CyaC as a template [2].
Through a genetic screen, we identified several activating mutations, that are involved in the regulation of CyaB by the Chp virulence system, and by solving the crystal structure of the CyaB catalytic domain, we can rationalize the effects of several of these mutations and suggest that CyaB employs regulation mechanisms similar to other Class III AC, but triggered by other stimuli. Our results reveal mechanistic insights into physiological and pharmacological regulation of CyaB and thus provide the basis for a better understanding of this signalling system and for exploiting it for drug development.
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[2] Steegborn et al, Nature Structural & Molecular Biology (2005) 12(1), 32-37

MPP038

The role of β1-integrin for Yop translocation in *Yersinia enterocolitica*

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Yersinia enterocolitica injects effector proteins (Yops) into host cells with a Type Three Secretion System (TTSS). Injection of Yops affects several cell functions what finally leads to immune evasion.
Former studies using cultured cells showed that an interaction of the *Yersinia* adhesion factors YadA and Invasin with β1-integrins on the host cell site acts as a prerequisite for Yop translocation. β1-integrins are transmembrane heterodimeric receptors which can switch between an active and inactive conformation, and trigger various signaling cascades inside the cell. In this study we want to show whether and how β1-integrin mediated signaling contributes to Yop translocation in vitro and discriminate between Inv and YadA triggered effects. For this purpose a β-lactamase reporter system was used to detect and quantify Yop injection in infected cells.
We will present evidence that YadA and Invasin show striking differences how they contribute to Yop injection.
(1) Monitoring Yop translocation into epithelial and fibroblastoid cells shows that Inv triggered Yop injection is always strictly dependent on the expression of β1-integrins and an intact β1-cytoplasmic domain. Thereby the β1-cytoplasmic domain seems to be crucial as a binding site for the adaptor talin in terms of inside-out activation and as a linker to the actin cytoskeleton. But it is not important as a transmitter for β1-integrin mediated outside-in signaling by the tyrosine kinases FAK, SRC or ILK. So a high affinity interaction between Inv and β1-integrin, in which inside-out activation by talin is involved, seems to be sufficient for Inv mediated effector translocation.
(2) In contrast, only in fibroblasts but not in epithelial cells interaction of YadA with β1-integrins is required for Yop translocation. Additionally the β1-cytoplasmic domain is only partly important for YadA triggered Yop injection. So depending on cell type β1-integrins are completely dispensible for YadA mediated Yop injection. This clearly demonstrates, that YadA can initiate Yop translocation also by so far unknown β1-integrin-independent mechanisms.

MPP039

Human formyl peptide receptor 2 senses and differentiates enterococci

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The human innate immune system counteracts bacterial invaders by multiple antimicrobial mechanisms including polymorphonuclear leukocytes (PMN), which represent the most efficient phagocytes first occurring at the site of infection.
The human formyl peptide receptor 2 (FPR2) is a seven-transmembrane G-protein coupled receptor and is found on various cells. Recently, we could show that FPR2 is crucial for recruiting and activating PMN in staphylococcal infections because it senses concentrations of the major staphylococcal cytolysins phenol-soluble modulin (PSM) peptides [1]. Moreover, FPR2 adjusts PMN responses with respect to PSM release and pathogenicity of staphylococcal species [2].
Enterococci represent another group of important nosocomial pathogens. In this study, we show that not only staphylococci but also certain enterococci are capable of producing ligands for FPR2 thereby underscoring the importance of this receptor in antibacterial host defense.
PMN chemotaxis and intracellular calcium influx were induced in a dose-dependent manner by supernatants of *Enterococcus faecalis* and *Enterococcus faecium*. Only for *E. faecium*, this effect could be inhibited by the *S. aureus*-derived FPR2-antagonist FLIPr. In agreement with this, calcium flux in receptor-transfected HL-60 cells showed that only *E. faecium* elicited a FPR2-specific response whereas *E. faecalis* did not. Also, vancomycin-resistant *E. faecium* isolates induced a considerably stronger response than vancomycin-susceptible isolates. However, both *E. faecium* and *E. faecalis* activated the FPR2 paralog FPR1, which senses bacterial formylated peptides. The enterococcal genomes do not encode peptides with apparent similarity to PSM peptides. To further characterize the unknown FPR2 ligands produced by *E. faecium*, supernatants were treated with proteases, which completely abolished the ability to stimulate FPR2 transfected HL-60 cells. This indicates that the unknown FPR2 ligands of *E. faecium* represent peptides.
In conclusion, we were able to demonstrate that certain enterococci produce peptide-derived microbial associated molecular patterns, which are sensed by human FPR2.
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[2] Rautenberg et al. (2011), Neutrophil responses to staphylococcal pathogens and commensals via the formyl peptide receptor 2 relates to phenol-soluble modulin release and virulence. *FASEB J*. Apr;25(4):1254-63.

MPP040

Protein-protein interaction within the Cpx-two component system

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Two-component signal transduction systems (TCS) are the main mechanisms by which bacteria sense and respond to environmental stimuli [1]. TCS typically consist of a sensor kinase (SK) and a response regulator (RR). The SK autophosphorylates upon detecting an inducing cue and transfers the phosphoryl group to its cognate RR which now promotes changes in cellular physiology or behavior [1]. To keep the TCS in balance, the RR gets dephosphorylated intrinsic or due to the phosphatase activity of the SK [1]. However, the mechanistic details about the precise signal integration and transfer remain still unknown [2].
The Cpx-envelope stress system is a well established TCS composed of the membrane-bound SK CpxA, the cytosolic RR CpxR and in addition of the accessory protein CpxP [3]. Factors that cause cell envelope stress as e.g. pH stress, salt stress and misfolded proteins induce the Cpx-TCS [3]. The accessory CpxP inhibits autophosphorylation of CpxA and supports the degradation of misfolded pilus subunits [3]. Previous functional and structural studies suggest not only that CpxP inhibits CpxA through a direct protein-protein interaction but also indicate how CpxP act as a sensor for misfolded pilus subunits, pH and salt [4]. With membrane-SPINE [5] and bacterial two-hybrid system, we were now able to demonstrate the direct physical protein-protein interaction between CpxP and CpxAin vivo. Furthermore, our data show under several Cpx-inducing conditions that CpxP is released from CpxA assigning CpxP as the sensor for specific Cpx-inducing stimuli. Release of CpxP from CpxA is assumed to result dimerization and consequently in the autophosphorylation of CpxA [1, 3]. Thus, our combined results lead to a deeper insight into the signal recognition in TCS in general.
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V.S. Müller, P.R. Jungblut, T.F. Meyer and S. Hunke, Proteomics (2011)11, p. 2124.

MPP041

Comparative secretome analysis of *Enterococcus faecalis* isolates from food and clinical origin

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The ubiquitous Gram-positive *Enterococcus faecalis* belongs to the group of lactic acid bacteria and is part of the natural gut microbiota of mammals, but is also found in a range of fermented foods, particularly in artisanal cheeses. The presence of *E. faecalis* in cheese can be considered beneficial, with its metabolic activity contributing to desired traits like texture or flavour. Furthermore, probiotic effects have been attributed to this organism. However, the widespread application of *E. faecalis* in starter

cultures for cheese fermentation or as a probiotic is currently limited by the potential health risks associated with its use. *E. faecalis* also occurs as an opportunistic pathogen that can cause severe infections such as endocarditis, septicemia and urinary tract infections. Therefore, thorough characterization of isolates is necessary in order to assess potential risks for susceptible individuals. In this study, we investigated the effect of the growth environment on the secretome of two phenotypically similar *E. faecalis* strains from food and clinical origin. To investigate the scenario of potentially pathogenic *E. faecalis* ingestion with food, they were grown in the standard laboratory medium M17 and in Simulated Colon Environment Medium (SCEM) to mimic the conditions in the gut. As many of the *E. faecalis* virulence factors identified so far are secreted, extracellular proteins were isolated, separated and identified by 1D-SDS-PAGE- LC-MS/MS and comparatively analyzed. A total of 346 proteins were identified. In the further analysis, special attention was given to known virulence factors as well as the 36 proteins being solely expressed in SCEM.

MPP042

A scavenger receptor on nasal epithelial surfaces - An important player in Staphylococcus aureus nasal colonization

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Many severe bacterial infections originate from the microflora of the host. One of the most frequent causes of such infections is Staphylococcus aureus, which colonizes the noses of about one third of the population. However, the molecular basis of this colonization is only understood incompletely. It has been demonstrated that cell wall glycoploymers (CWGs) are important for adhesion of Gram-positive bacteria to host cells. The cell wall teichoic acid (WTA) of S. aureus has been shown to mediate adhesion to nasal epithelial cells and to be crucial for S. aureus colonization in a cotton rat model. However, the appropriate receptor on nasal epithelial cells remains elusive.
Recent research in the field of glycobiology suggests members of the scavenger receptor family as an interaction partner for WTA. Previously this hypothesis could be confirmed by using inhibitors against scavenger receptors, which inhibited adhesion of S. aureus to nasal epithelial cells, markedly. Recently, the expression of a scavenger receptor on epithelial cells has been described. In accordance, function blocking antibodies to this receptor inhibited S. aureus adhesion to human epithelial cells under static and mild sheer stress conditions. To further elucidate these findings in a nasal colonization model in cotton rats we established primary cell cultures of nasal epithelial cells from cotton rats. Thereby we could detect the expression of the mentioned scavenger receptor. Moreover, we were able to demonstrate a specific binding of WTA to these primary cotton rat epithelial cells using WTA labeled latex beads. Recently, we confirmed the crucial role of this scavenger receptor in vivo by blocking S. aureus adhesion to nasal epithelial cells by preincubating nasal epithelia of cotton rats with an antibody against this scavenger receptor. Thus, we here present the first receptor for WTA in nasal colonization.

MPP043

Infection of human endothelial progenitor cells with *Bartonella henselae* induces vessel-like growth *in vitro*.

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Endothelial progenitor cells (EPCs) are a heterogeneous mixture of adult stem cells that play an essential role in revascularization after vascular damage. Their discovery over a decade ago led to various pre-clinical and clinical trials investigating the use of these cells in regenerative medicine for ischemic injury. In our work we investigated an unconventional method of improving the angiogenic potential of EPCs through bacterial infection.*Bartonella spp*.are facultative intracellular pathogens and the only known bacteria to induce angiogenesis in humans. Here we describe for the first time the course of a bacterial infection of EPCs with the vasculotropic bacterium*B. henselae*. Our data demonstrate that EPCs are highly susceptible *toB. henselae*and that infection does not disturb their initial differentiation under angiogenic conditions. Upon infection EPCs show a strong activation of hypoxia inducible factor-1 (HIF-1), the key transcription factor in angiogenesis. This is followed by the signature HIF-1-dependent pro-angiogenic cell response including production of cytokines such as vascular endothelial growth factor (VEGF) and adrenomedullin (ADM). Furthermore,*B. henselae*prevents apoptosis of EPCs and induces cell migration along a stromal cell-derived factor (SDF)-1 gradient, both essential functional components of the angiogenic response. Finally, when culture plates are coated with a basement membrane which simulates the extra-cellular matrix(*Matrigel*™), infected EPCs assemble into complex vessel-like structures*in vitro*. We have recently shown that heat-killed*B. henselae*can also induce the building of vessel-like structures*in vitro*suggesting the involvement of some yet-unknown outer membrane element. Cumulatively, our data demonstrate that infection with*B. henselae*can improve the angiogenic capacity of EPCs and induce vessel-like growth*in vitro*. At present we are working to phenotypically and genetically characterize the transformation of EPCs from circulation progenitor cells to vessel-like structures and identify genes and pathways involved in this bacterial induced process.

MPP044

Sweet toothed bats without cavities - almost no appearance of dental caries in the frugivorous bat *Artibeus jamaicensis*

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Dental caries is a widespread disease which affects humans and other mammal species, but obviously not the frugivorous bat *Artibeus jamaicensis*. There are many studies concerning dental decay in humans and animal models, but so far little is known about the complex microbiological and environmental interactions which lead to dental caries. Although these bats consume nearly exclusively figs and consequently high amounts of sugars, they are less affected by cavities than humans. To confirm this observation and to offer an explanation, a study was conducted including ecological, microbiological, dental, and microscopical techniques.
Animals were captured in the wild during field work on Barro Colorado Island (Panama). The teeth of the bats were analyzed with dental criteria to determine the incidence of dental caries. Only three of 230 captured *A. jamaicensis* were affected. In general, only 0.9 % of the surveyed surface of the teeth showed appearance of dental plaque as documented by staining, notoriously less than in humans.
To identify the oral microbial community of these bats, saliva samples were taken, genomic DNA was extracted, and the amplified bacterial 16S rDNA fragments were analyzed by 454-Pyrosequencing. It was found that the oral microbiota of healthy bats is similar to human saliva regarding the composition of microorganisms with one exception: Healthy bats saliva lacks obligate anaerobic bacteria. Nevertheless, plaque-forming as well as facultative anaerobic bacteria could be found. Obligate anaerobes could only be detected in a saliva sample of a caries affected *A. lituratus*. All anaerobic bacteria found are potentially cariogenic under anaerobic conditions, normally found in elder dental plaque. The confirmation of potentially cariogenic bacteria in the saliva of bats leads to the assumption that there are no substances protecting against caries in saliva inhibiting their growth.
Extracted teeth of dead specimens were examined in reference to human teeth in their hardness, surface structure, and enamel. First results show a smoother surface structure, the lack of pores, and a thinner enamel layer. These results indicate that it is the particular surface shape of the enamel of the teeth of bats which is related to less caries incidence in *A. jamaicencis*, despite the attendance of cariogenic bacteria.

MPP045

Staphylococcal major autolysin (Atl) is involved in excretion of cytoplasmic proteins

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In both gram-positive and -negative bacteria as well as in yeasts typical cytoplasmic proteins/enzymes are found outside the cell in the culture supernatant or attached to the cell surface where they may contribute to virulence. Nothing is known how these “extracellular” cytoplasmic proteins are translocated through the cytoplasmic membrane and this type of secretion was referred to as "nonclassical protein secretion". We could demonstrate that in *Staphylococcus aureus* the major autolysin Atl plays a crucial role in release of cytoplasmic proteins. We could show that in *Staphylococcus aureus* 20 typical cytoplasmic proteins were excreted and using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a cytoplasmic indicator enzyme, we showed that all clinical isolates tested excreted this protein. To answer the question of how discriminatory the excretion of cytoplasmic proteins is, we performed a two-dimensional PAGE of cytoplasmic proteins isolated from WT. We disproved the common opinion that only highly expressed and abundant cytoplasmic

proteins are excreted. On the contrary, the most abundant cytoplasmic proteins were not found in the secretome. These results suggest that there exists a selection mechanism in the excretion of cytoplasmic proteins. The presence or absence of prophages had little influence on the secretome pattern. Furthermore we could show in the *atl* mutant that secondary peptidoglycan hydrolases were increased both in the secretome as well the corresponding genes were transcriptionally up-regulated suggesting a compensatory mechanism for the atl mutation. As the major autolysin binds at the septum site, we assume that the proteins are preferentially released at and during septum formation.

MPP046

Relaxed substrate specificity of bacterial phospholipid flippases - alanyl- phosphatidylglycerol confers wild type level daptomycin resistance in the presence of lysyl-phosphatidylglycerol flippases in *Staphylococcus aureus*

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The Multiple Peptide Resistance Factor (MprF) of *Staphylococcus aureus* is a bifunctional enzyme with two separable functional domains that synthesize positively charged lysyl- phosphatidylglycerol (Lys-PG) and facilitate Lys-PG flipping into the outer leaflet of the membrane, resulting in repulsion of cationic antimicrobial peptides encountered during colonization and infection of the human host or competing microorganisms (Peschel *et al*.,2001, Ernst *et al*., 2009). The impact of MprF- mediated Lys-PG production on CAMP resistance has been confirmed with MprF homologs from major human pathogens, such as *Listeria monocytogenes*, *Bacillus anthracis, Mycobacterium tuberculosis*, and also with MprF homologs from *Rhizobium tropici* and *Bacillus subtilis*. Interestingly, some MprF proteins synthesize zwitterionic alanyl-phosphatidylglycerol (Ala-PG), such as MprF homologs from *Enterococcus faecium*, *Clostridium perfringens*, or *Pseudomonas aeruginosa*. The Impact of the production of zwitterionic Ala-PG on susceptibility to antimicrobial peptides has so far only been studied in the gram- negative pathogen *P. aeruginosa*, which alanylates 6% of the phospholipids, leading to select phenotypes, such as reduced susceptibility to cromium ions, protamine sulphate and cefsulidin (Klein *et al*., 2009). We expressed the Ala-PG producing MprF of *C. perfringens* in a *S. aureus mprF* deletion mutant and show that Ala-PG integrates effectively in the phospholipid biosynthetic pathways of *S. aureus*, leading to the production of more than 60 % Ala-PG. The production of Ala-PG in *S. aureus* enabled us to investigate the impact of zwitterionic Ala-PG on CAMP susceptibility in a gram positive pathogen and led to the unexpected observation that Ala-PG is as effective in conferring a basic level of resistance to the CAMP- like antibiotic daptomycin, as Lys-PG, as long as Lys-PG flippases are present, indicating that Lys-PG flippases have broad range specificity for aminoacyl- phospholipids.

MPP047

Functional genome analysis of *Paenibacillus larvae*, the causative agent of the American Foulbrood of honey bees (AFB)

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Paenibacillus larvae is a rod-shaped and spore-forming Gram-positive bacterium causing American Foulbrood of honey bees. First *P. larvae* has been described as *Bacillus larvae* in 1906. Recently, it was shown that the species *P. larvae* comprises different genotypes differing in virulence at the individual insect and at the colony level [1]. *P. larvae* is able to infect honey bees and honey bee larvas via the spores, but only kills the latter. The way of infection and killing is still poorly understood. It has been shown, that approximately 10 infectious spores from virulent strains are sufficient to cause mortality [2].
Raw-sequencing of the *P. larvae* str. 08-100 (ERIC I) and str. 04-309 (ERIC II) genomes were done by using 454-pyrosequencing. The obtained sequences were assembled and analyzed. Subsequently, contigs were sorted and remaining gaps closed. The genome size of *P. larvae* str. 04-309 (ERIC II) and the GC content are approximately 4.05 Mb and 45 %, respectively, while the genome size of *P. larvae* str. 08-100 is about 4.51 Mb and has a GC content of 44 %. The annotation of the genome sequences provided new important insights into genes involved in pathogenesis.

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MPP048

Transcriptome and proteome analyses of *P. aeruginosa* PAO1 expressing the biofilm-inhibiting SDR BpiB09 reveal a significant effect on QS-controlled genes

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In *Pseudomonas aeruginosa*, quorum sensing-regulated gene expression contributes to the formation and maintenance of biofilms and their tolerance to conventional antimicrobials. Therefore QS and QS-related gene expression are promising targets for the development of new antimicrobial drugs. Here we report on a genome wide transcriptome analysis using next generation sequencing RNA-seq and proteome analysis of PAO1 cells expressing the recently published novel and metagenome-derived short-chain dehydrogenase/reductase (SDR) BpiB09[1]. Expression of BpiB09 resulted in a significantly reduced pyocyanin production, decreased motility, poor biofilm formation and decreased paralysis of nematodes. HPLC-MS analyses correlated these phenotypes with the almost complete absence of synthesized autoinducers in PAO1. Our genome wide comparative transcriptome and whole-cell-protein proteome analysis of *P. aeruginosa* PAO1 expressing BpiB09 identified significant effects on most of the quorum sensing controlled genes like *lasI*, *rhlI*, *pqsR* and *pqsABCD*. A least 38 of these well-known QS-regulated genes were strongly (>10-fold) down-regulated in their expression profiles. As well a significant number of genes and ORFs were detected that had been linked to QS-phenotypes in PAO1 and that were less than 10-fold but at least 4-fold altered in their expression level. Altogether these were 80 genes/ORFs and among those we found the *hcnB* and *hcnC* genes involved in hydrogen cyanide synthesis, the *aprD* and *aprE* genes involved in alkaline protease secretion as well as *lecB* and *lasA*. Additionally a defined subset of so far not QS-linked genes was affected. These data were supported by 2D-proteome analyses of PAO1 cells. Altogether, our data suggest that the direct expression of SDR in PAO1 and/or the exogenous addition of BpiB09 to growing PAO1 cells have profound effects on PAO1 gene expression and might be a useful tool for the development of novel anti-biofilm strategies.

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MPP049

Comparative global transcriptome analysis ofCandida albicansandCandida dubliniensisallows new insights into chlamydospore development

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Candida albicans and Candida dubliniensisare highly related pathogenic yeast species displaying differences in their epidemiology and in some phenotypic characteristics, including virulence-associated traits. During in vitro growth on certain nutrient-poor media, both share the species-specific ability to produce chlamydospores, large spherical, thick-walled cells with unknown function. Interestingly however, onlyC. dubliniensisforms pseudoyphae with abundant chlamydospores on Staib agar (syn.Guizotia abyssinicacreatinine agar), on whichC. albicansgrows as a budding yeast. In order to get new insights into chlamydospore development, we compared the global transcriptional profile of both species during growth in Staib medium by DNA microarray analysis and RNA sequencing. As a means to narrow down the putative set of chlamydospore- versus pseudohyphae-specific genes, the analysis of aC. albicansnrg1mutant was also included in this study.C. albicansmutants in this global repressor of filamentation have previously been demonstrated to produce not only pseudohyphae but also abundant chlamydospores in Staib medium, similar asC. dubliniensis. At present, individual identified genes are functionally characterized inC. albicansandC. dubliniensis, for their putative role in chlamydospore development but also with respect to other phenotypic

characteristics. These studies should contribute to a better understanding of the fundamental biology of these medically important pathogenic fungi.
Staib P, Morschhäuser J (2005) Differential expression of theNRG1repressor controls species-specific regulation of chlamydospore development inCandida albicansandCandida dubliniensis. Mol Microbiol 55: 637-652

MPP050

The immune modulatory zwitterionic cell wall polymer of *Staphylococcus aureus* - an important role in CA-MRSA pathogenicity?

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Staphylococcus aureus is a major pathogen, in both nosocomial and community-acquired infections that can cause a large variety of infections but skin and soft-tissue infections (SSTIs) are the most common type caused by CA-MRSA. The pathogenicity of CA-MRSA strains seems to depend on an array of different virulence factors; however the relative activity of these factors is still unclear. Recently, we demonstrated that the cell wall polymer WTA (wall teichoic acid) of *S. aureus* is a major modulator for the early phase of abscess formation [1]. The immune modulatory activity of WTA depends on its zwitterionic character and the ability to stimulate CD4+ T-cell proliferation in an MHC II-dependent manner [2], which is in contrast to the current dogma a non peptide antigen. We found that highly pathogenic CA-MRSA strains exhibit an elevated amount of WTA in their cell wall. Purified protein-free cell wall fractions from CA-MRSA induce T-cell proliferation and cytokine production more efficiently than cell wall from non CA-MRSA. Thus, cell wall fractions of CA-MRSA strains are more active in skin abscess formation, which can be attributed to the higher WTA amount in their cell wall. Hence, up-regulation of WTA expression is one of the possible mechanisms CA-MRSA exploit to gain virulence. To confirm our hypothesis, we currently elucidate a detailed expression profile of important structural genes of WTA biosynthesis by quantitative real-time PCR. Therefore, selected CA-MRSA strains will be compared to different non CA-MRSA strains in vitro and ex vivo using a skin abscess model in mice. Furthermore, we plan to measure the expression of teichoic acid biosynthesis enzymes on the protein level. Our goal is to get more insights into the regulatory elements involved in WTA biosynthesis. This study may contribute to a better understanding of the complex pathology of SSTIs caused by highly virulent CA-MRSA strains.
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MPP051

Cell contact dependent virulence gene expression in *Yersinia pseudotuberculosis*

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The enteropathogenic bacterium *Yersinia pseudotuberculosis* colonizes the human gut and transmigrates through the mucosal cell layer into underlying lymphatic tissues and organs. This causes several gut- and lymph- associated diseases and in rare cases autoimmune diseases.
As *Y. pseudotuberculosis* can be found in the environment as well as inside its host's body, it needs to perfectly adapt to these particular conditions. Especially virulence genes are tightly environmentally regulated. We show that *Y. pseudotuberculosis* senses cell contact to distinguish between environment and host and to adapt gene expression. Especially genes required in the late phase of infection (yop regulon) seem to be upregulated upon contact to human cells.
Within this work the impact of cell contact on the expression of the outer membrane protein YadA and its transcriptional regulator LcrF was investigated. During infection YadA mediates adhesion to and invasion into epithelial cells and helps to evade host's immune system. Monolayers of epithelial cells (HEp-2) were infected with *Y. pseudotuberculosis* carrying *yadA* and *lcrF* promoter reporter gene fusions to *GFP* or *luciferase*. The expression pattern of bacteria in contact to cells were compared to free bacteria and analyzed by fluorescence microscopy, luminescence detection or western blotting. We could show that the expression of *yadA* is directly activated through a cell contact dependent expression of its regulator *lcrF*. Further, CsrA, a RNA-binding protein of the carbon storage system, is part of this cell contact sensing cascade. The importance of several other factors could be excluded.
By analyzing various mutants and performing microarray analysis we want to identify more participating factors and the cell contact sensor.

MPP052

Expression of filamentous hemagglutinin in *Bartonella henselae*

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The gram-negative, zoonotic pathogen *Bartonella henselae* causes cat scratch disease and vasculoproliverative disorders. In recent years, two essential pathogenicity factors of
B. henselae have been investigated in detail: the trimeric autotransporter adhesin *Bartonella* adhesin A (BadA) and the VirB/D4 type IV secretion system (VirB/D4 T4SS). Analysis of the genomic sequence of *B. henselae* gave evidence for an additional pathogenicity factor, the filamentous hemagglutinin (FHA). Eight genes of different length encode homologues of filamentous hemagglutinin (FhaB), and four genes encode homologues of FhaC/HecB of *Bordetella pertussis* forming potentially a two partner secretion system. Until now, nothing is known on the role of FHA in infections with *B. henselae*. Here, we analyzed the expression of *fhaB* and *fhaC/hecB* in two different *B. henselae* strains (Marseille, Hoston-1) under different growth conditions (different pH values, at 30 and 37 °C) by quantitative realtime-RT-PCR. Our data revealed that *fhaB* and *fhaC/hecB* were (i) expressed in both *B. henselae* strains and (ii) expression was pH-dependent meaning that the expression level increased with increasing pH values. Cultivation temperature did not have an effect on expression. These results give first evidence, that filamentous hemagglutinin is in fact expressed in *B. henselae* and might therefore play a role in *Bartonella* infections.

MPP053

Determination of intracellular survival of *Streptococcus agalactiae* in the interaction with monocytic and granulocytic cells

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*Streptococcus agalactiae (*Group B Streptococci, GBS) is an important cause of human invasive infections in newborns, pregnant women and immunocompromised adult patients. The ß-hemolysin of GBS is a surface associated toxin and regarded as a major virulence factor of GBS. It is regulated by the *cov* two-component regulatory system, which controls numerous virulence factors of GBS. To determine the role of the ß-hemolysin for intracellular survival and to rule out the effect of other virulence factors controlled by *cov*, we investigated hemolytic and nonhemolytic GBS mutants for intracellular survival in primary human granulocytes and THP-1 cells.
We examined the role of ß-hemolysin for interaction with the monocytic and granulocytic cells using a serotype Ia *S. agalactiae* wild type strain and an isogenic nonhemolytic deletion mutant of this strain. Both strains were fluorescently labeled with an EGFP expressing plasmid. Following infection of eukaryotic cells with GBS, the intracellular bacteria were evaluated by FACS analysis and culturing of intracellular bacteria. Interestingly, the non-hemolytic mutants were able to survive in the intracellular environment in significantly higher numbers than the hemolytic strain. A finding that was observed for primary granulocytes as well as for THP-1 cells. To exclude the possibility that the observed differences in survival were due to host cell death induced by the hemolytic but not the non-hemolytic strain, Lactate Dehydrogenase (LDH) assays were carried out and confirmed a better survival capacity of the nonhemolytic strain. To assess the induction of IL-8 following infection with GBS, ELISA determinations were performed. While a considerable release of IL-8 could be observed, we could however not find a significant difference in their ability to induce the chemokine. To determine the bacterial mediators of IL-8 release in this setting, cell wall preparations from both strains were incubated with THP-1 cells. Both preparations were found to exert a potent proinflammatory stimulus on THP-1 cells. In conclusion our results indicate, that the *S. agalactiae*ß-hemolysin has a strong influence on the intracellular survival of GBS and that a tightly controlled regulation of ß-hemolysin expression is required for the successful establishment of GBS in different host niches.

MPP054

BopC is a type III secretion effector protein of *Burkholderia pseudomallei*

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Question: *Burkholderia pseudomallei* is the causative agent of melioidosis, the disease endemic in southeast Asia and northern Australia. The promising component causing pathogenesis is the Bsa type III secretion system (T3SS). Only two Bsa-secreted effectors have been conclusively identified to date. Here we explored the T3SS-dependent secretion and the virulence mechanism of a *B. pseudomallei* putative effector protein BopC (BPSS1516), which is encoded from the *bpss1516* gene adjacent to its putative chaperone *bpss1517*.
Methods: *B. pseudomallei bopC* gene was cloned as Glutathione S-transferase (GST)-tagged constructs and expressed in *Escherichia coli*. Pull down and co-purification assays were conducted to address the interaction between BopC and its putative chaperone BPSS1517. Translocation assay was performed to investigate the importance of the N-terminal amino acids of BopC. *B. pseudomallei bopC* mutant was constructed by insertion mutagenesis. A549 lung epithelial cells were employed for invasion assay.
Results: Immunoblotting demonstrated that BopC was secreted into culture supernatant by the wild-type *B. pseudomallei* strain, but its secretion was abolished in the *bsaZ* T3SS mutant, suggesting that BopC is secreted *via* T3SS. Pull down and co-purification assays confirmed that BopC interacts with its putative chaperone, BPSS1517, *in vitro*. The first 20 N-terminal amino acids of BopC were found to be sufficient to mediate the T3SS-dependent translocation of a reporter protein from a heterologous enteropathogenic *E. coli* host into mammalian cells. Finally, *B. pseudomallei bopC* mutant was found to be less invasive than the wild-type strain in the epithelial cells.
Conclusions: *B. pseudomallei* BopC is a newly identified type III effector protein. The secretion of BopC is dependent on Bsa T3SS. Furthermore BopC is implicated in the *B. pseudomallei* invasion into epithelial cells.

Muangman S, Korbsrisate S, Muangsombut V, Srinon V, Adler N L, Schroeder G N, et al., BopC is a type III secreted effector protein of *Burkholderia pseudomallei*. FEMS microbiology letters, 2011;**323** (1): 75-82.

MPP055

Proteomic characterization of the different *Legionella pneumophila* life stage

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The Gram-negative bacterium *Legionella pneumophila* is the causative agent of a severe and often fatal human pneumonia, Legionnaires' disease. In the natural environment, *L. pneumophila* inhabits freshwater and biofilms and parasitizes protozoan hosts. The intracellular life cycle of *L. pneumophila* is divided into two distinct stages: the replicative phase (RP), where the bacteria multiply until the nutrients cease, and the transmissive phase (TP), where the bacteria render virulent and invasive. Upon prolonged periods of stress (such as nutrient deprivation, temperature change, etc.), *L. pneumophila* may enter into the viable but not culturable (VBNC) state where the bacteria only show a very low level of metabolic activity and do not grow on standard media. Remarkably, VBNC state *L. pneumophila* may resuscitate and thereby regain culturability as well as virulence after passage through a eucaryotic host. In consequence, VBNC state *L. pneumophila* have to be considered as a public health hazard. To characterize the distinct stages of life for *L. pneumophila*, in this study we performed a systematic proteomic comparison of broth-grown RP and TP and stress-induced VBNC states. To induce VBNC cell formation in *L. pneumophila* bacteria, different stress conditions like cold and heat stress, nutrient limitation, and several chemical agents were tested. During heat stress (42°C), the number of CFU decreased to zero within 68 days whereas the microcosms remained stable with respect to culturability at 4°C and 21°C for at least 140 days. Despite the drastic decrease in CFU counts, 40% of the bacteria remained viable according to microscopic live/dead analysis. For proteome analysis, it is essential to separate the VBNC-*Legionella* from dead bacteria. To this purpose, we used fluorescence-activated cell sorting (FACS). Our work will contribute to a deeper understanding of the modification processes within bacteria in response to different conditions, including adaptation to long-term stress.

MPP056

Carolacton cause inhibition of *Streptococcus mutans* biofilms through the serine/threonine protein kinase PknB

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Biofilm forming bacteria are often significantly more resistant to drug treatments than their planktonic counterparts and are associated to various pathological conditions in humans as e.g. cystic fibrosis, colonisation of indwelling medical devices and dental plaque formation. Therefore new substances and therapies aiming to erase biofilms are urgently needed. One possible strategy to cope with this demand is to disturb signal-transduction in biofilms.
Carolacton, a secondary metabolite isolated from the myxobacterium *Sorangium cellulosum* was proven to disturb biofilm viability at nanomolar concentrations. Treated biofilms showed a leakage of cytoplasmic content (proteins and DNA) in growing cells at low pH. Using a ß-galactosidase reporter strain and quantitative PCR the efflux-dynamics of intracellular proteins and DNA were quantified. The strong acidification occurring during biofilm growth was shown to be responsible for the biofilm-specific activity of carolacton.
A chemical conversion of the of the ketocarbonic function of the molecule to a methlyester did not impact its activity, indicating that carolacton is not functionally activated at low pH by a change of its net charge. Beside multiple genes involved in cell wall metabolism the VicKRX and ComDE two-component signal transduction systems were found to play an essential role in the cellular response to carolacton treatment as identified by time-resolved microarray analysis. The influence of carolacton on *de novo* cell wall metabolism and cell division was further investigated by fluorescence microscopy using a fluorescent vancomycin derivative.
A sensitivity testing of mutants with deletions of all 13 viable histidine kinases and the serine/threonine protein kinase PknB identified only the Δ*pknB* mutant to be insensitive to carolacton treatment. Furthermore a strong overlap between the PknB-regulon in *S. mutans* and the genes affected by carolacton treatment was found. In conclusion the data suggest that carolacton interferes with PknB-mediated signalling in growing cells. The altered cell wall metabolism and architecture cause membrane damage and cell death at low pH.

MPP057

Systems biology analysis of metabolic adaptation of *Staphylococcus aureus* and analysis of the impact of protein complexes

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Systems biology approaches, combining modern OMICs techniques with bioinformatics and mathematical modeling allow us to explore how adaptation of cell physiology and metabolic processes affect pathogenicity in a more "panoramic view" and thus may accomplish a new level of understanding. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains causing serious infections even in healthy individuals (CA-MRSA) represents a major threat and underscores the need for a comprehensive understanding of virulence mechanisms. Since it is known that the basic cell physiology determines not only growth but pathogenicity as well, we want to identify the concentrations and complex formation of proteins involved in central carbon metabolism. Two physiological adaptation scenarios, which are very likely encountered by *S. aureus* during infection settings are addressed *in vitro*: i) changes in the supply of carbon sources and ii) the aerobic/anaerobic shift. In addition, protein concentrations of internalized *S. aureus* cells by non-professional phagocytes will be determined.
For absolute quantification of proteins we use the QconCAT technology which allows quantification of up to 15 proteins within a synthetic standard protein. The design as well as the construction (heterologous expression, labeling with stable isotopes, purification and quality control) of 4 QconCAT proteins in total (covering all enzymes of the glycolysis, TCA-cycle, gluconeogenesis, pentosephosphate pathway and proteins involved in the aerobic/anaerobic shift) is completed. For experimental screening of protein complexes we use *in vivo* cross-linking and tagging experiments. Enzymes of the central carbon metabolism are used as a bait-protein, tagged with a Strep-tag and inserted into the plasmid pMAD

which is then used to exchange the wild type copy of the gene with the tagged gene version.
Finally, integrating these isotope and tap-tagged data into bioinformatic approaches allows us not only to reconstruct fluxes but also the dynamics of different protein complexes. Consequently, we will determine to what degree protein complexes are required for physiological fitness of *S. aureus*, which accounts to a better understanding of its pathophysiology.

MPP058

The Patatin-like Protein VipD/ PatA - a phospholipase A of *Legionella pneumophila* playing a role in bacterium-host interaction

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The phospholipase VipD/ PatA is one of eleven patatin-like proteins (PLP) found in *Legionella pneumophila*. Patatin-like proteins are lipid-acyl hydrolases mainly characterized in plants so far, but they were previously shown to be widely coded within bacterial genomes. In *L. pneumophila*, VipD/ PatA was determined to be a substrate of the type IVB secretion system by Shohdy et al. (PNAS 2005). We focused on this protein because it is the *L. pneumophila* PLP which is most similar to ExoU, a potent phospholipase and cytotoxin of *Pseudomonas aeruginosa* that causes rapid host cell death upon injection by the type III secretion system of this pathogen. We previously found that similar to ExoU, VipD/ PatA localizes to the cytoplasmic membrane after expression in A549 lung epithelial cells. Here, the C-terminal region of the protein plays an essential role, because deletion of the 129 C-terminal amino acids abolishes proper targeting. We now aimed to characterize the protein determinants for translocation of VipD/ PatA to the cytoplasmic membrane. Therefore, we mutated or deleted potential phosphorylation sites, special conserved motifs and a potential transmembrane domain as well as a region of low complexity to evaluate the influence of these parts for membrane localization. The lipolytic activity of VipD/ PatA, for which Serin-72 embedded in a G-X-S-X-G lipase motif is essential, is not required for membrane targeting.

MPP059

sRNA-mediated control of the primary invasion factor invasin in *Yersinia pseudotuberculosis*

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Yersinia pseudotuberculosis is an enteric human pathogen that causes gut-associated diseases. The primary virulence determinant is the outer membrane protein invasin. This protein mediates bacterial binding to and invasion through the epithelial cells of the gut.
Invasin expression is controlled by rovA (regulator of virulence A) in response to the surrounding temperature and ion availability (1). One key regulator of rovA in turn is the csr (carbon storage regulator) system. It is composed of the RNA-binding protein CsrA and two regulatory RNAs, csrB and CsrC. These RNAs sequester CsrA thus controlling its function (2). Expression and stability of the two RNAs is controlled by different regulators and sensory cascades. Recent findings showed that CsrB expression is activated upon bacterial contact to epithelial cells. Although a two-component regulator system is known to induce CsrB synthesis the cell contact signal is not integrated via this sensor system.
The regulation of CsrC involves various transcriptional and post-transcriptional modulators. For example the Yersinia modulator A (YmoA) confers CsrC RNA stability. However, CsrC stability is not directly mediated by YmoA. Microarray analysis indicated that YmoA affects expression of different RNases, which might control CsrC turnover.

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MPP060

Proteomic characterization of host pathogen interaction during internalization of *S. aureus* by A549 cells

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S. aureus was widely considered an extracellular pathogen. In the last years it became evident that it is able to invade and persist in non-professional phagocytic cells [1]. Besides milder skin infections this Gram-positive bacterium is known to induce severe systemic infections like sepsis or endocarditis [2]. Therefore, it is of interest to understand the mechanism of adaptation of the pathogen upon infection as well as the response of its host. Proteomic studies of internalized bacteria are strongly limited by the low number of cells recoverable from the host. With our newly developed workflow that combines a pulse-chase SILAC approach, GFP supported enrichment of bacterial proteins by fluorescence activated cell sorting (FACS) and gel-free mass spectrometry analysis (MS), it is possible to monitor the proteome of *S. aureus* RN1HG pMV158GFP internalized by S9 cells, human bronchial epithelial cells [3]. We identified about 600 *S. aureus* proteins from 3-7x10^6 internalized bacteria and more than 500 could be quantified. A further interesting host model for this infection assay is the A549 cell line. Those cells belong to the alveoli of human lungs, produce surfactant [4] and secrete certain cytokines and have therefore an impact also on the innate immune system. In present study we analyzed the proteome of *S. aureus* after internalization by A549 cells. During a time range from 1.5-6.5 hours after infection 1-3x10^6 bacteria could be separated from the host cells. With an optimized protocol identification and quantification of 842 proteins could be accomplished. We could show that proteins belonging to e.g. peptidoglycan biosynthesis and glycolysis/gluconeogenesis were upregulated during infection. However, staphylococcal virulence factors which have an influence on its pathogenicity like hemolytic toxins, adhesins and enzymes which interfere with host cell signaling are mainly secreted into the host cell lumen and therefore lost during FACS sorting. In order to make those extracellular proteins also accessible, we now established methods of enriching cellular components in which *S. aureus* presumably resides [5]. Using density gradient centrifugation and lipobiotin attached to magnetic beads, compartments containing *S. aureus* and its secreted proteins were isolated and analyzed by LC-MS. Microscopic techniques were applied to prove the intracellular localization of *S. aureus*. Compartments containing internalized *S. aureus* will then be isolated and lysed probably making the *in vivo* secretome of *S. aureus* accessible to proteome analysis.

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MPP061

Direct activation of *Legionella pneumophila* glycerophospholipid: cholesterol acyltransferase PlaC by the zinc metalloproteinase ProA

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Legionella pneumophila infects both mammalian cells and environmental hosts, such as amoeba. Enzymes secreted by *Legionella pneumophila*, such as phospholipases A (PLA) and glycerophospholipid: cholesterol acyltransferases (GCAT), may target host cell lipids and therefore contribute to Legionnaires' disease establishment. *L. pneumophila* possesses three proteins, PlaA, PlaC, and PlaD, belonging to the GDSL family of lipases / acyltransferases. Enzymatic activity of these enzymes depends on a conserved nucleophilic serine embedded into the GDSL motif as well as on the residues aspartate and histidin together building up the catalytic triad. The sequences of PlaA and PlaC harbour N-terminal signal peptides for Sec and subsequent type II-dependent protein export, whereas the secretion mode of PlaD is still unclear. PlaC is the major GCAT secreted by *L. pneumophila* and able to transfer free fatty acids from phospholipids to cholesterol and ergosterol, additional to PLA and LPLA activities. This GCAT activity is post-transcriptionally regulated by ProA, a secreted zinc metalloprotease. Since cholesterol is an important compound of mammalian cell membranes and ergosterol of amoeba membranes, GCAT activity might be a tool for host cell remodelling during *Legionella* infection. Our aim was to characterize the mode of PlaC GCAT activation and to determine how ProA processes PlaC. Our results indicate that PlaC forms two protein loops due to intramolecular disulfide bonds which are both essential for PLA / GCAT activities. Analyses of the potential cleavage site as well as loop 1 deletion mutants suggest the importance of ProA loop deletion for GCAT activation. Our data therefore indicate a novel enzyme inhibition / activation mechanism where loop 1 displays an inhibitory effect on PlaC GCAT and full PLA activity until PlaC is exported to the external space and subsequently activated by ProA.

MPP062

Invasiveness of Salmonella serotypes Typhimurium and Enteritidis for transfected primary chicken intestinal cells.

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Introduction: 33% of salmonellosis is caused by S.Enteritidis. Human contamination mainly occurs from poultry products. S. Enteritidis represents a major problem in chicken rearing because infection is insidious in these animals and they are usually asymptomatic carriers of Salmonella. Bacteria pass to the eggs transovar and probably also retrocecal and chickens contaminate their fellow birds by spreading Salmonella in the environment via excretion. S. Typhimurium is localized in the host cell within a membrane compartment known as the Salmonella-containing vacuole (SCV). These bacteria are capable to survive and replicate within the SCV, eventually killing the host cell and being released into the extracellular medium to infect other cells. Salmonella serotypes are responsible for gastroenteritis in humans in industrialized countries [1] [2].
Method: The intestine wall from 18-day-old chicken embryos was cut, washed several times and digested for 18h at 4°C and subsequently for 30 min at 37°C with Trypsin/EDTA. Cells were finally separated by gently pipetting in the presence of DMEM supplemented with 2% chicken serum, 2 mM L-glutamine and 10 μg/ml epithelial growth factor and incubated for 2h at 37°C [3]. After incubation cells were diluted in basal seeding medium supplemented with 10% FCS and 2mM L-glutamine. Transfection of primary chicken cells was carried out using retroviral vector pBABE-hTERT-p53DD. The cells were cloned twice by limiting dilution and characterized using epithelial cell markers. An invasion assay was carried out to research the invasiveness of Salmonellafor primary chicken intestinal cells. Cells were infected with Salmonella for 1 hour (multiplicity of infection (MOI) 100). Serial dilutions were plated for viable bacterial counting. For quantification of intracellular bacteria chicken cells were treated with gentamicin to kill extracellular bacteria. After incubation epithelial cells were lysed and the number of internalized bacterial cells was enumerated by plating serial lysate dilutions [4].
Results: This study shows that Salmonella does not cause apoptosis in cultured primary chicken epithelial cells and is able to infect the cells and divide within them after 3-4 hours of infection.

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MPP063

Characterization of the C-terminal half of major cell-associated phospholipase A PlaB of *Legionella pneumophila*

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The lung pathogen *Legionella pneumophila* expresses a variety of phospholipases potentially involved in disease-promoting processes and development of pneumonia. The recently identified major cell-associated phospholipase A (PLA)/ lysophospholipase A (LPLA) with an additional hemolytic activity, designated PlaB, shares no homology to previously described phospholipases. So far, it was shown that PlaB utilizes a typical triad of Ser-Asp-His for effective hydrolysis of phospholipids located within the N-terminal half of the protein for cleavage of phospholipids, such as phosphatidylglycerol (PG) and -choline (PC) as well as the respective lysophospholipids. We further determined that PC- but not PG-hydrolyzing PLA activity is directly linked to the hemolytic potential of PlaB. The first characterized member of a new family of lipases also plays an important role as virulence factor in a guinea pig infection model. Until now, the function of the C-terminal half of the protein is unknown, but it contributes to lipolytic activity. Therefore we aimed to characterize its function. Interestingly, the analysis of three C-terminally truncated versions of PlaB recombinantly expressed in *E. coli* revealed, that a lack of only 5 amino acids (aa) leads to a decrease of PC-PLA activity. The lack of 10 aa at the C-terminus however results in a decrease of PG- and PC-PLA activity whereas the removal of 15 aa completely abolishes the enzymatic activity. Furthermore, sufficient amounts of soluble and active PlaB have been successfully purified and used for antibody production. This now allows detection of the protein in *L. pneumophila* and thereby characterization of its definite localization and export pathway.

MPP064

Resistance phenotypes mediated by Aminoacyl-Phosphatidylglycerol Synthases

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The specific aminoacylation of the phospholipid phosphatidylglycerol (PG) with alanine or with lysine catalyzed by aminoacyl-phosphatidylglycerol synthases (aaPGS) was shown to render various organisms less susceptible to antibacterial agents. This study makes use of *Pseudomonas aeruginosa* chimeric mutant strains producing lysyl-phosphatidylglycerol (L-PG) instead of the naturally occurring alanyl-phosphatidylglycerol (A-PG) to study the resulting impact on bacterial resistance. Consequences of such artificial phospholipid composition were studied in the presence of an overall of nine antimicrobials (β-lactams, a lipopeptide antibiotic, cationic surfactants, CAMPs) to quantitatively assess the effect of A-PG substitution (with L-PG, L-PG and A-PG, increased A-PG levels). For the employed *P. aeruginosa* model system an exclusive 'charge repulsion mechanism' does not explain the attenuated antimicrobial susceptibility due to PG modification. Additionally, the specificity of nine orthologous aaPGS enzymes was experimentally determined. The newly characterized protein sequences allowed to establish a significant group of A-PG synthase sequences which were bioinformatically compared to the related group of L-PG synthesizing enzymes. The analysis revealed a diverse origin for the evolution of A-PG and L-PG synthases as the specificity of an individual enzyme is not reflected in terms of a characteristic sequence motif. This finding is relevant for future development of potential aaPGS inhibitors.

MPP065

Yada mediated complement evasion of *Yersinia enterocolitica*

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The Yersinia adhesin A (YadA) is a trimeric autotransporter adhesin with multiple functions in host-pathogen interactions. A major function of YadA is the protection of Yersinia from killing by the host complement system. Numerous studies have shown that YadA is essential for complement evasion in vitro (1,2,4). Recently, we have generated several Yersinia enterocolitica (Ye) strains expressing point mutated versions of YadA carrying a single amino acid exchange (G389 was exchanged with amino acids of increasing side chain size: A, S) within the membrane anchor domain. We have shown in vitro that when expressed in E. coli the YadA trimer stability decreases with increasing side chain size (3). We have also shown that the decreased trimer stability has an effect on serum resistance, interaction with the complement regulator Factor H and virulence of Yersinia enterocolitica in a mouse model of infection (5). To further dissect the mechanism of YadA-mediated complement evasion we thoroughly analysed binding of complement factors and found that YadA of Yersinia enterocolitica is able to actively recruit the complement protein C3. C3 that binds to the bacterial surface is subsequently inactivated by complement factors in a YadA-dependent manner. The binding of C3 also plays a supportive role in the binding of the complement regulator Factor H. C3 inactivation results in a reduced activation of the later steps of the complement system, especially in a reduced formation of the terminal complement complex. Consequently, this leads to an enhanced serum resistance of Yersinia enterocolitica.

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MPP066

RNA-sequencing analysis of c-di-GMP effects on the UPEC-transcriptome

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The bacterial signaling molecule cyclic dimeric GMP (c-di-GMP) is a key factor controlling the transition from acute infection into a state of chronic infection. Generally, high intracellular levels of c-di-GMP favor persistence and interfere with acute virulence. However, little is known about the molecular mechanisms underlying this c-di-GMP based switch from virulence to persistence. In particular, only few mRNAs or non-coding RNAs are known to change their cellular abundance in response to

altered c-di-GMP levels. Here we used a comparative RNA-sequencing approach to identify genes in uropathogenic E. coli that are controlled by c-di-GMP. In addition we used ribo-sequencing to globally map transcription start sites and to discover several novel transcripts, including predicted regulatory RNAs. Results are discussed with a focus on genes encoded in pathogenicity islands and on additional factors known to be involved in E. coli virulence during bladder infections.

MPP067

Posttranslational protein modifications in host pathogen interactions

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Many bacteria cause life threatening diseases and face clinicians with a dilemma caused by an increasing resistance of bacterial infections against current treatment options. Protein modifications, such as phosphorylation and thiol oxidation, seem to be crucial for the host in order to react adequately to the invading pathogen. In pathogenic bacteria, on the other hand, they are believed to be critical for adaptation and virulence. Our group hypothesises that analysing protein modifications in host pathogen models with system wide approaches will not only lead to a better understanding of the biology of infections but can also result in the development of novel pharmaceutical intervention strategies. To this end, we use quantitative mass spectrometry based proteomics in combination with modification specific enrichment and visualization strategies including affinity-based kinase and phosphopeptide enrichments as well as fluorescence and differential chemical thiol-redox labelling.

MPP068

Microevolution of *Pseudomonas aeruginosa* clonal lineages

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Question: Chronic airway infections with *P. aeruginosa* are frequent in individuals with cystic fibrosis (CF) and are a paradigm of how environmental bacteria can conquer, adapt and persist in an atypical habitat. The phenotypical conversion goes along with a microevolution of the genome during colonization of the airways.
Methods: At Hannover Medical School sequential isolates have been collected since the onset of colonization from patients seen at the CF clinic, who became chronically colonized in their airways with *P. aeruginosa* between 1982 and 1991. Detecting the same clonal lineage for isolates over a long time course by a genotyping microarray indicated the persistence and adaptation of the respective *P. aeruginosa* clone. In order to analyse microevolution we selected sequential isolates for comparative genomics. First, intermediary and late isolates were sequenced by Illumina sequencing and analysed for their global fitness and phenotypic diversity. Informative genetic markers were then used for genotyping all sequential isolates to reconstruct the temporal evolution within the clonal lineages during the infection periods of up to 20 years.
Results and Conclusion: Results for isolates belonging to the common clone PA14 for example indicated a diversification into three branches in the patient's lungs with 15 nucleotide substitutions and a large deletion acquired by the population during the observation period. The genome of another common clone (clone C) remained invariant during the first three years, 15 years later, however, 947 transitions and 12 transversions had accumulated in an isolate, likely after acquisition of a mutation in *mutL*.
Another study on intraclonal diversity of P. aeruginosa was done on CF isolates belonging to the clonal complex TB. The respective strains were isolated in 1983 in the Hannover CF clinic from different patients and showed highly divergent phenotypes. Sequencing, however, revealed only few genomic differences as only a few nucleotide exchanges and small deletions were detected as probable causes for divergent phenotypes.

MPP069

Differential attachment of Lyme disease spirochetes to human keratinocytes

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Upon entry into the human host by a bite of an infected tick, spirochetes have developed diverse strategies to successfully colonize host tissues and survive in an unfavorable hostile environment. Attachment to human cells is thought to be a key step for the establishment of an infection that causes multiple clinical symptoms including serious neurological as well as long-term dermatological manifestations. Infection of the various tissues depends on the ability of spirochetes to bind to different cell types. In this study, we examined the ability of Lyme disease spirochetes belonging to five distinct human pathogenic genospecies (*Borrelia burgdorferi*, *B. afzelii*, *B. garinii*, *B. spielmanii*, and *B. bavariensis*) and two genospecies with disputed pathogenic potential (*B. lusitaniae* and *B. valaisiana*) to bind to human keratinocytes. Among the genospecies analysed, *B. valaisiana* and *B. spielmanii* showed the strongest attachment (up to 1200 borrelial cells per 100 keratinocytes) while *B. bavariensis*, *B. garinii*, and *B. afzelii* displayed a moderate binding activity (up to 120 bacterial cells per 100 keratinocytes) suggesting a role of these five genospecies in mediating infection of human skin. In contrast, *B. burgdorferi* and *B. lusitaniae* completely lacked binding. Furthermore, intraspecies differences have also been observed among *B. garinii*, *B. bavariensis*, *B. afzelii*, and in particular *B. valaisiana*.
Recently, it has been shown that bacterial proteins involved in serum resistance confer attachment to human keratinocytes. Among Lyme disease spirochetes, complement regulator-acquiring surface proteins (CRASPs) are known to be essential for resistance to killing by human serum. To further assess the role of these infection-associated outer surface proteins for mediating interaction to human cells, *B. garinii* cells producing distinct CRASPs originally derived from *B. burgdorferi* were employed. Interestingly, binding capacity to human keratinocytes increased up to four-fold when employing *B. garinii* producing CRASP-4 but not CRASP-1, CRASP-2 or CRASP-3 compared to wild-type *B. garinii* cells lacking CRASPs. Taken together, these data provide evidence that distinct borrelial genospecies differ in their ability to attach to human keratinocytes and, in addition, support a role of certain CRASPs as adhesins of Lyme disease spirochetes.

MPP070

The role of insertion elements in the evolution of antibiotic resistance of *Staphylococcus aureus*

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In recent years the pathogen *Staphylococcus aureus* was able to acquire resistance to nearly all antibiotics used in clinical practice. Exposure to subinhibitory concentrations of these antibiotics may lead to an induction of mutational mechanisms, as for example the SOS response (1) or the mobilization of IS elements. IS elements are mobile genetic elements that are able to alter gene expression in staphylococci by integration into different regions of the chromosome which may result in an inactivation or overexpression of genes. Our experiments are focused on the mechanisms involved in the regulation of the transposition of IS elements, such as IS*256* and IS*257*, and their influence on vancomycin resistance development in *S. aureus*. It has previously been reported that the treatment with subinhibitory concentrations of different groups of antibiotics resulted in an activation of the transposition frequency of the insertion element IS*256*r in *S. aureus* HG001 (2). Furthermore, the alternative sigma factor B was shown to be a negative regulator of the transposition activity of IS*256*r by generating an antisense RNA of the transposase (2,3). Additionally, we identified the *rsbU* gene, which encodes a positive regulator of sigma factor B, as a hotspot for IS*256* insertion in *S. aureus*. The *rsbU*::IS*256* insertion mutants displayed a white colony colour as a consequence of inhibition of staphyloxanthin biosynthesis and appeared preferentially in the presence of antibiotics and after incubation at 45°C. In further experiments we will test the effect of the IS*256* insertion into *rsbU* on the transposition frequency of IS*256*.
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MPP071

Phenotypic and genotypic characteristics of Vibrio isolates from environmental, clinical and seafood samples in Germany

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The number of infections caused by pathogenic Vibrio spp. worldwide has steadily increased in recent years. Vibrio spp. are ubiquitous Gram-negative bacteria found naturally in marine and estuarine waters, including aquaculture settings. They are a leading cause of seafood-borne bacterial illness. Increasing incidence of vibrioses in marine animals and humans has been linked to rising seawater temperature due to global warming and the growing global trade of seafood. The systematic assessment and characterisation of Vibrio spp. pathogenic to humans in environment, seafood and disease in Germany is a major focus of the research program VibrioNet. In the present study, phenotypic and genotypic traits of sets of isolates of *V. parahaemolyticus* and *V. vulnificus* from different sources were comparatively evaluated to assess their pathogenic potential. Molecular typing using Multi-Locus Sequence Typing and virulence markers were used to evaluate the genetic profiles and virulence potential. Phenotypic virulence characteristics were addressed using tests for pathogenicity related traits such as serum resistance, hemolysin production, motility, biofilm formation and extracellular enzyme activities.

Finally, selected strains were characterized inin vitro screens using cell cultures. First results of these investigations are presented.

MPP072

Genetic and morphological analyses of the vancomycin and daptomycin resistant *Staphylococcus aureus* strain VC40

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Glycopeptide antibiotics (e.g. vancomycin) are the mainstay of therapy for serious infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, MRSA strains with reduced susceptibility to glycopeptides have emerged during the last decade. In times of increased antibiotic treatment failure, there is an obvious need to understand how bacteria respond to the presence of antimicrobial compounds and develop resistance. In order to study the impact of an elevated mutation frequency on vancomycin resistance development, we had previously generated the highly vancomycin resistant strain *S. aureus* VC40 (MIC: 64 µg/ml) by serial passage of *S. aureus* RN4220Δ*mutS* (MIC: 2 µg/ml) in the presence of increasing concentrations of vancomycin (1).
In the current study, cross-resistance to daptomycin, a lipopeptide antibiotic recently introduced for the treatment of complicated skin and skin structure infections (cSSSI) caused by MRSA, was observed in strain VC40. To further elucidate the resistance phenotype of *S. aureus* VC40, the full genome sequences of this strain and its parent strain RN4220Δ*mutS* were determined using 454 sequencing technology. A total of 79 mutations in genes related to cell wall metabolism, transport and gene regulation were detected in strain VC40. Point mutations were also found in the histidine kinases of the two-component regulatory systems VraSR and YycFG (WalKR), which significantly impact on the biosynthesis and turnover rates of the bacterial cell wall (2,3), and accompanying transcriptome analyses indeed showed an altered expression of affected regulons in strain VC40. Further morphological analyses using transmission electron microscopy revealed that strain VC40 was characterized by an abnormal cell envelope morphology that may result from deregulated VraSR or YycFG systems. Reintroduction of the VraS mutations into the parental background led to a significant increase in resistance against several cell wall-active antibiotics, including vancomycin, daptomycin and the lantibiotic mersacidin. In conclusion, characterization of strain VC40 reveals a central role for VraS mutations in resistance development to cell envelope-active agents and may help to gain a better understanding of the mode of antibiotic resistance evolution in *S. aureus*.
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(2) Kuroda et al. 2003, Mol Microbiol 49(3):807-821
(3) Dubrac et al. 2008, Mol Microbiol 70(6):1307-1322

MPP073

Antibiotic acyldepsipeptides inhibit bacterial cell division by inducing the ClpP peptidase-dependent degradation of the cell division protein FtsZ

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A novel class of antibacterial acyldepsipeptides (ADEPs) exerts prominent activity against Gram-positive bacteria including multi-resistant *Staphylococcus aureus in vitro* and *in vivo* [1]. ADEPs act by dysregulating ClpP peptidase of the bacterial caseinolytic protease system. Usually, the activity of ClpP is tightly controlled by ATP-dependent Clp-ATPases and accessory proteins. ADEPs overcome these tight control mechanisms, switching ClpP from a regulated to an uncontrolled protease that predominantly targets unfolded or flexible proteins as well as nascent polypeptides in the absence of Clp-ATPases [1,2,3]. Although the activity of ADEPs can be explained on the molecular level of its target ClpP, the specific events that finally lead to bacterial cell death remained unknown.
In our study, we investigated the effect of ADEP treatment on different Gram-positive species using high-resolution microscopy. In the presence of low inhibitory ADEP concentrations, the coccoid cells of *S. aureus* and *Streptococcus pneumoniae* swelled to more than 3-fold the volume of wild type cells, and the rod-shaped cells of *Bacillus subtilis* grew into very long filaments, which reached 60- to 100-fold the length of untreated cells, clearly indicating stalled bacterial cell division. To gain further insights into the underlying molecular mechanism, we followed the events that led to the inhibition of cell division. We observed that ADEP treatment resulted in the inhibition of septum formation in *S. aureus* and *B. subtilis*, while chromosome segregation was rather unaffected. Localization studies with GFP-labeled cell division proteins revealed that the ADEP-ClpP complex interferes with key components of early cell division and therefore perturbs normal divisome formation. By analyzing cell extracts of ADEP-treated bacteria, immunoblotting revealed that treated cells showed a significantly decreased abundance of the essential FtsZ protein, which consequently ends in bacterial cell death [4]. Specific degradation of FtsZ by ADEP-activated ClpP was confirmed by *in vitro* studies using purified ClpP protein. ADEPs demonstrate that beside their interesting antibacterial potency they are excellent tools to examine central mechanism of bacterial physiology, like cell division and regulated proteolysis.
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[2] Kirstein et al. 2009, EMBO Mol. Med. 1: 37-49
[3] Lee et al. 2010, Nat. Struct. Mol. Biol. 1787: 1-8
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MPP074

Molecular mode of action of acyldepsipeptide antibiotics in mycobacteria

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Acyldepsipeptides (designated ADEPs) are a novel class of antibiotics, which act through an unprecedented mechanism by dysregulating the bacterial caseinolytic protease ClpP [1], which is otherwise controlled by Clp-ATPases and adapter proteins. Binding of ADEPs to ClpP prevents the interaction of the peptidase with corresponding Clp-ATPases and leads to the inhibition of all natural functions of ClpP [2]. Additionally, ADEPs induce opening of the entrance pore to the proteolytic chamber, which confers independent proteolytic activity to the peptidase. ADEP-activated ClpP degrades nascent polypeptides at the ribosome and flexible proteins in the absence of Clp-ATPases [3]. Recently, it has been shown that FtsZ, an essential cell division protein, is a particularly sensitive target for ADEP-activated ClpP [4].
Although ADEPs demonstrated promising antibacterial activity against staphylococci, streptococci and enterococci *in vitro* and in infection models, their development was hampered by the fact that ClpP is not strictly essential in these genera and prone to mutation. To this end, one of our aims is to identify pathogens, which are less susceptible for mutations and therefor have the potential for a slower development of ADEP resistance. A special feature of mycobacteria is that they encode two chromosomal copies of ClpP [5] and recent observations have shown that both genes are essential in these organisms [6, 7]. In this study, we are focusing on the efficacy of ADEPs against mycobacteria. We demonstrate that ADEPs are active against *Mycobacterium bovis* BCG, which is closely related to *M. tuberculosis*, a pathogen of global importance and the causative agent of tuberculosis. We further observed that ADEPs activate the ClpP complex of *M. tuberculosis in vitro* to degrade several model substrates including the flexible protein casein. Thus, ADEPs are ideal tools to study the function of these unique ClpP proteins in mycobacteria. Furthermore, ClpP represents a promising new drug target due to its essentiality in these organisms, and ADEPs are interesting lead structures for the development of new anti-tuberculosis drugs.
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[6] Sasetti et al. 2001, PNAS, 98: 12712-12717
[7] Ollinger et al. 2011, J. Bacteriol., epub. ahead of print.

MPP075

Will not be presented!

MPP076

Interference of quinoles and aminocoumarines regarding RecA mediated response in *Staphylococcus aureus*

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Different gyrase inhibitors bind to different moieties of the gyrase both resulting in arrest of DNA replication. The chinolones are known to induce the bacterial SOS response through the generation of double strand breakes resulting in RecA activation. RecA dependent cleavage of the LexA repressor results in error prone repair, which favours mutations and

therefore resistance development. Other gyrase inhibitors, namely the aminocoumarines bind the GyrB subunit which leads to competitive inhibition of the ATPase activity of gyrase but not to double strand brakes. Here we observe partially antagonistic effects of quinolones (ciprofloxacin) and aminocoumarines (novobiocin) with regard to RecA induction, SOS response, mutation rate and phage induction in the human pathogen *Staphylococcus aureus*.
Site-specific mutants (*recA*, *lexA*) as well as an inducible *recA* mutant were constructed and the combined action of gyrase inhibitors analysed by transcriptional analysis and Western blots. In addition effects on phage induction and mutation frequencies were assessed.
We could show that ciprofloxacin results in a RecA dependent derepression of LexA target genes such as the error prone polymerase SACOL1400. In contrast the aminocoumarine novobiocin leads to a decrease in RecA expression on protein as well as transcript level. Interestingly, the combination of ciprofloxacin and novobiocin results also in decrease of RecA. However, by combination of both antibiotics although RecA expression is significantly repressed the SOS response is still induced as shown by the induction of the LexA target gene coding for the error-prone polymerase SACOL1400. Also phage induction was not altered by RecA repression. An artificially dose-dependent recA expression system showed us, that induction of the lexA genes as well as phage induction is clearly correlated to the RecA expression level.
In summary, the result indicate that there are additional RecA independent mechanisms involved in lexA autocleavage induced by a mix of ciprofloxacin and novobiocin.To identify this second activator or pathway is very important, since it is involved in generating resistant bacteria and needs to be considered during antibacterial therapy.

MPP077

Comparative proteome analysis of *Staphylococcus aureus* strains co-internalized into S9 cells

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Staphylococcus aureus, the cause of a wide spectrum of severe community-acquired and nosocomial infections, is acknowledged as an intracellular pathogen, as it can be internalized and persist in non-professional phagocytic cells in cell culture experiments [1]. During the internalization process, *S. aureus* has to adapt to the intracellular environment to survive or even persist within the host, but still little is known about these adaptive changes on proteome level. *S. aureus* virulence factors, which are important to establish an infection are tightly controlled by global regulators. The accessory gene regulator (*agr*) is one of the major global regulators of *S. aureus* virulence. RNAIII, the effector molecule of the *agr* system, positively controls the production of exoproteins and negatively controls cell surface bound proteins during the post exponential growth phase [2]. There is evidence that this regulatory system plays a role in the establishment of an infection and host cell killing. For instance, expression of *agr* is initially increased in the acute phase of infection in non-professional phagocytic cells [3].Furthermore, Δ*agr* mutants are attenuated in their virulence in several mouse models [4,5,6,7].
The aim of this study is to comparatively investigate the adaptive and competitive response of *S. aureus* HG001 wild type and its isogenic Δ*agr* mutant upon co-internalization by human bronchial epithelial cells (S9). The striking advantage of such a co-infection assay is that both strains are internalized simultaneously and adapt to the host under exactly the same conditions.
Proteome analysis of the co-internalized *S. aureus* are performed with the well established workflow, which combines a classical infection assay with high capacity cell sorting and gel-free proteomics [8].
To make the internalized Staphylococci accessible, they have to be separated from debris of lysed S9 cells and distinguished between wild type and mutant by FACS. After validation, the fluorescent marker gpCerulean of the Δ*agr* mutant showed a clear distinction to the GFP expression of the wild type. Accordingly, we are now able to sort co-internalized *S. aureus* parallel in distinct wells of a 96-well plate.
In conclusion, with this setting we are able to monitor co-internalized HG001 wild type and Δ*agr* mutant and make them with FACS-sorting and on membrane digest accessible for proteome analysis.

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MPP078

Comparative dRNA-seq analysis of multiple *Campylobacter jejuni* strains

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Campylobacter jejuni, a Gram-negative spiral-shaped Epsilonproteobacterium, is one of the most common causes of bacterial gastroenteritis in humans [1]. While it is a commensal of chicken, it has also been associated with the development of autoimmune disorders like Guillain-Barré and Miller-Fisher syndromes in humans. The microaerophilic, foodborne pathogen is able to survive under various stress conditions imposed by the environment and the host. The small genome of *C. jejuni* (1.65 Mb) carries only a few transcriptional regulators and almost nothing is known about the role of non-coding RNAs in this pathogen. Like the related human pathogen *Helicobacter pylori*, *C. jejuni* also lacks the RNA chaperone, Hfq, which plays a pivotal role in sRNA-mediated regulation in many bacteria.
Massively parallel cDNA sequencing (RNA-seq) has been revolutionizing transcriptome analysis in both eukaryotes and prokaryotes and has revealed a wealth of novel information about microbial transcriptomes [2]. Recently, we have developed a novel differential approach (dRNA-seq) selective for the 5' end of primary transcripts, which revealed an unexpectedly complex transcriptional output and massive antisense transcription from the small and compact genome of the related Epsilonproteobacterium *H. pylori* [3]. This method allowed us to define a genome-wide map of transcriptional start sites (TSS) and operons, and revealed more than 60 sRNAs including potential regulators of cis- and trans- encoded mRNAs in *H. pylori*.
Here we present a comparative dRNA-seq approach to analyze the transcriptome structure and TSS conservation of four different *C. jejuni* strains. This comparative study reveals that the majority of TSS is conserved among all strains but that there are also several strain-specific TSS indicating divergent transcription patterns among different strains. Moreover, Northern blot analysis confirmed similar and differential expression patterns of several conserved and strain specific sRNA candidates in *C. jejuni*. This is the first comparative analysis of the primary transcriptomes and sRNA repertoire of multiple *C. jejuni* strains and will provide new insights into riboregulation in this bacterial pathogen.

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MPP079

How a thioredoxin-like protein influences the susceptibility to ß-lactam antibiotics in *Staphylococcus aureus*.

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As a human pathogen, *Staphylococcus aureus* is capable of colonizing the hostile ecological niche of the anterior nares in humans and has therefore developed different strategies in order to survive during various environmental stresses. During the process of infection, *S. aureus* is exposed to multiple antimicrobial compounds such as oxidative burst products and antibiotics. The underlying regulatory pathways governing susceptibility or resistance are complex and still remain only superficially understood. Within this tightly balanced resistance network a thioredoxin-like protein YjbH has been shown to control disulfide stress response in *Bacillus subtilis* by monitoring the controlled degradation of the transcriptional stress regulator Spx via the proteasome-like ClpXP protease. Similar functions could be attributed to the *S. aureus* YjbH homolog using the disulfide stress-inducing agent diamide as in *B. subtilis*. Further experiments revealed the indispensable role of conserved cysteine residues within the YjbH protein for this activity. In addition, inactivation of YjbH led to moderate resistance to oxacillin and other β-lactam antibiotics, which was associated with an increase in peptidoglycan cross-linking and higher penicillin-binding protein 4 levels. Of note, the impact of YjbH on β-lactam susceptibility was still observed when the conserved cysteines of YjbH were mutated indicating that the roles of YjbH in disulfide stress and β-lactam resistance rely on different types of

interactions. Taken together, our results indicate that the ClpXP adaptor YjbH may have more target proteins than previously thought and that a close link between oxidative burst and β-lactam resistance mechanisms may exist in *S. aureus*.

MPP080

Fully human antibodies targeting crucial proteins of *Staphylococcus aureus*

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The opportunistic pathogen *Staphylococcus aureus* is a serious health threat causing a wide range of infections with often fatal outcome. The emergence of antibiotic resistant strains, most important the methicillin resistant strains (MRSA), has further complicated the treatment of *S. aureus* infections.
In order to develop a novel therapy, we have employed MorphoSys' HuCAL® antibody library, which as of today has delivered more than 70 therapeutic lead candidates being developed in various indications and thousands of research antibodies. Previous antibody-mediated therapies for *Staphylococcus* infections targeted known virulence factors or capsule antigens. Our new approach focuses on different essential membrane proteins crucial for the survival of *S. aureus* (including MRSA).
Fully human antibodies were selected from the HuCAL® Platinum library binding to synthesized peptides representing extracellular loops of the target proteins. The antibodies were shown to bind to *S. aureus* cells by ELISA and FACS. Functional data of the antibodies from *in vitro* assays will be presented.

MPP081

Prevalence of enteropathogens in suckling piglets with diarrhoea in German organic farms with special regard to the role of *Clostridium perfringens* type A

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Piglet diarrhoea is a multifactorial disease, which induces relevant economic losses in piglet production due to piglet loss, impaired growth, and treatment costs. In this study 699 faecal samples of diarrhoeic piglets in 258 litters from 18 organic farms were investigated for the occurrence of enterotoxigenic *Escherichia coli* (ETEC), *Clostridium* (*Cl.*) *perfringens*, Rotavirus, and Coccidia. Additionally 369 faecal samples of sows and 419 samples of healthy piglets were investigated for *Cl. perfringens*.
In 39.5% of all diseased litters *Cl. perfringens* type A was detected, the most frequent enteropathogen in this study. 89.7% of *Cl. perfringens* type A isolates were tested positive for the gene coding β2-toxin. Rotavirus occurred in 27.6%, and Coccidia in 20.0% of the diseased litters, whereas the diagnosis ETEC was made in an unexpected low number of cases (7.7%). *Cl. perfringens* type C was not found in any sample. Remarkably the detection rate of *Cl. perfringens* type A among healthy suckling piglets reached 58.9%, which is was even higher than in diarrhoeic piglets. Only 8.5% of *Cl. perfringens* type A isolates from sows carried the gene for β2-toxin, which could be detected in 94.2% of all suckling piglet isolates (healthy as well as diarrhoeic). This discovery implicates an overestimation of the role of sows as source of *Cl. perfringens* infection for suckling piglets in the past.

MPP082

Bacillus – extremely fast *C. elegans* killer: insights into mechanisms of virulence

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C. elegans has been proven as a comprehensive model system to study mechanisms of microbial pathogenicity. We employed this system in order to carry out systematic analysis of nematode-*Bacillus* interactions. Our survey showed (Rae et al. 2010) that majority of *Bacillus* strains are benign to nematodes and only 3% of more then 800 *Bacillus* strains tested showed virulence to *C. elegans*. One of the virulent strains – *B. sp. 27*, kills *C. elegans* extremely fast (16 hours) and represents one of the fastest *C. elegans* killers known today; therefore we decided to study virulent mechanisms of this strain in more detail. Using transposon mutagenesis, we found a number of *B. sp. 27* mutants with attenuated virulence to *C. elegans*. Interestingly, many genes that were hit in transposon mutants appeared to be plasmid-encoded, suggesting that virulent genes may be encoded by plasmids. To confirm that, we generated several plasmid-cured derivatives of *B. sp. 27*. Killing assay with *C. elegans* showed that those derivatives lost virulence completely, confirming that virulent factors of *B. sp. 27* are indeed plasmid-encoded. Taking into account that *B. sp. 27* was identified as *B. thuringiensis*, we checked if wild type and plasmid-cured derivatives produce Cry toxins (parasporal crystals). We found that in contrast to wild type, plasmid-cured derivatives no longer form parasporal crystals, strongly suggesting that Cry toxins are responsible for *C. elegans* killing. Currently, we are doing whole genome sequencing of *B. sp. 27* in order to find out what genes are encoded by virulent plasmids and to identify type of Cry toxin that kills nematodes. On the other hand, we use all advantages of *C. elegans* as a model to reveal molecular mechanisms of nematode defense response against toxic *B. sp. 27*.

MPV1-FG

Modulation of phosphoinositide metabolism by *Legionella spp*

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The opportunistic pathogensLegionella pneumophilaandLegionella longbeachaeemploy a conserved mechanism to replicate in amoebae and macrophages within a unique compartment called the "Legionella-containing vacuole" (LCV). Formation of LCVs requires the bacterial Icm/Dot type IV secretion system which, for *L. pneumophila*, translocates more than 250 "effector proteins" into the target host cell. The *L. pneumophila* effect or proteins SidM and SidC anchor to the phosphoinositide (PI) lipid phosphatidylinositol-4-phosphate (PI(4)P) on the cytosolic face of LCVs, where they interfere with host cell vesicle trafficking and signal transduction [1]. Pulldown experiments with PI lipids coupled to agarose beads revealed that *L. longbeachae* SidC also specifically binds to PI(4)P.
L. pneumophilamodulates the PI pattern of LCVs in an Icm/Dot dependent manner, yet the mechanism remains elusive. We recently discovered a bacterial PI phosphatase, which is translocated into the host via the Icm/Dot T4SS and preferentially hydrolyses poly-phosphorylated PIs yielding PI(4)P. This PI phosphatase, termed LppA, may have a function in regulating the PI pattern of the LCV during infection.
The genetically tractable social amoebaDictyostelium discoideumhas been used in a number of studies to analyze LCV formation of *L. pneumophila*. The PI 5-phosphatase OCRL1 and itsDictyosteliumhomologue Dd5P4 localize to LCVs, restrict intracellular bacterial growth and are implicated in retrograde trafficking [1]. Using specific GFP-fused PI probes heterologously produced inDictyostelium, LCVs were found to accumulate distinct PI lipids. Current efforts aim at a detailed characterization of the LCV PI pattern and the role of PI-modulatingLegionellaeffector proteins.
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MPV2-FG

The YycFG (WalRK/VicRK) two-component regulatory system of *Staphylococcus aureus* and its capability to sense changes in membrane fluidity

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Two-component regulatory systems (TCSs) play a major role in bacteria and confer the ability to recognize and to respond to changes in their environment. TC systems are composed of a sensor histidine kinase and a cognate response regulator. After sensing of a particular signal the kinase undergoes autophosphorylation and in a subsequent step the phosphoric group is transferred to the response regulator, changing its ability to bind DNA and thereby also affecting transcriptional expression of target genes.
In the prominent nosocomial grampositive pathogen *Staphylococcus aureus*, YycFG (WalRK/VicRK) represents an outstanding TCS, characterized by its essentiality [1] and important role in maintaining cell wall homoeostasis [2], especially after localization to the cell division site [3]. Whilst knowledge on localization and regulatory activity of this system has been steadily increasing in the last years, less is known about the particular signals, which modulate the YycG kinase activity. In order to address this question, the full-length YycG wild type kinase - including both transmembrane (TM) domains - and a mutant version with an aa exchange (Y306N) in the cytoplasmic PAS domain, have been expressed as C-His_6-tagged proteins and tested in two *in vitro* systems. The first one utilizes Triton X-100 as a membrane mimicking surfactant and in the second system the kinases were reconstituted in a membrane provided by phospholipid-liposomes. After suitable conditions for YycG autophosphorylation and phosphoric group transfer to YycF had been determined, it could be demonstrated that high alkali salt concentrations, in particular of KCl, and low temperatures were necessary to stimulate YycG activity. Further examination indicated that in both systems increased salt concentrations in combination with low temperatures seemed to lead to a decreased fluidity of micelles and liposomes, indicating that a certain stiffness is necessary for activity. In case of the mutated YycG(Y306N) kinase the effect of changes in the microenvironmental condition were less pronounced, leading to an increased activity in general. Since it was shown that the full-length kinase can be turned off by a decrease in viscosity, we

suggest that the YycG kinase might *in vivo* respond to membrane fluidity via its TM domains.

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MPV3-FG

Structure and function of the PorB porin from disseminating *N. gonorrhoeae*

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The outer membrane of Gram-negative bacteria is permeabilized by a large number of porin channels for the uptake of small molecules. Porins are the major outer membrane proteins of proteobacteria and mitochondria. Some porins of the *Neisseriaceae* clade can insert and permeabilize the inner mitochondrial membranes of mammalians cells during infection and may lead to mitochondrially induced apoptosis. Porins of the serotype A ($PorB_{IA}$) of *N. gonorrhoeae* are associated with disseminating gonococcal disease and mediate the rapid invasion into host cells in a phosphate sensitive manner. To understand these functions on the basis of structural data we analyzed the structure of $PorB_{IA}$ isolated from wildtype *N. gonorrhoeae*. The structure in complex with ATP and phosphate solved at the resolution 3.1 Å displays a surplus of positive charges inside the channel with a potential for substrate transfer. ATP is coordinated by positively charged residues via aromatic, sugar and pyrophosphate moiety atoms. A short b-bulge inserted into the b2-strand nearby the location of ATP and the long L3 loop narrows the barrel diameter significantly and together support substrate specificity. Phosphate ions known to interfere with bacterial uptake after host cell contact are present, one of which is coordinated by two Arg residues nearby the ATP at the extraplasmic channel exit. *In vivo* assays of bacteria carrying mutations at residues complexing phosphate molecules confirm the importance of these residues for host cell invasion. Interestingly, the structure also comprises a small peptide sequence as remnant of a periplasmic protein which physically links porin molecules to the peptidoglycane layer. Although similar in sequence to related *Neisseriacea*, $PorB_{IA}$ shows differences which more clearly deviate from the non-apoptotic porin from *N. sicca* or *N. lactamica*. Models of these PorB channels are compared in structure and possible functional implications are outlined and tested.

MPV4-FG

Methicillin Resistance inStaphylococcus aureusDepends on ß-O-GlcNAcylation of Wall Teichoic Acids

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Staphylococcus aureuspeptidoglycan is densely functionalized with anionic polymers called wall teichoic acids (WTAs), which are required for proper cell division. Preventing WTA polymer synthesis sensitizes methicillin resistantS. aureus(MRSA) to beta-lactams. Here we describe the discovery and biochemical characterization of a novel glycosyltransferase, TarS, that attaches b-O-N- Acetylglucos-amine (b-O-GlcNAc) residues toS. aureusWTAs. We show that b-O-GlcNAcylation of WTAs is required for the beta-lactam resistant phenotype in MRSA. The b-O-GlcNAc residues play a specific structural role in mediating resistance since neither a-O-GlcNAc modifications nor b-O-glucosyl modifications confer resistance. We propose that b-O-GlcNAcylated WTAs scaffold peptidoglycan biosynthetic complexes containing the resistant transpeptidase PBP2a. The b-O-GlcNAc transferase identified here, TarS, is a new target for inhibitors that sensitize MRSA to beta-lactams.

MPV5-FG

Identification of β-haemolysin encoding genes in *Streptococcus anginosus*

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Streptococcus anginosus is a commensal of the oral cavity, the gastrointestinal and the female urogenital tract. It has clinical significance in abscess formation and has been suggested to play a pathogenic role in patients with cystic fibrosis. An interesting feature of these bacteria is the inconsistent phenotype regarding Lancefield antigens as well as haemolytic activity. While a considerable percentage of *S. anginosus* strains display a prominent ß-haemolytic phenotype, the corresponding genes have not been identified yet. In different streptococcal species the ß-haemolysin is a modified short peptide (SLS) that is related to Class I bacteriocins. It is encoded in the *sag* gene cluster including genes for the corresponding posttranslational modifications and transport machinery.
By random chromosomal integration of the pGhost9:IS*S1* transposition vector we generated a plasmid-based mutant library of the haemolytic *Streptococcus anginosus* strain ATCC 12395. This library was screened for mutants showing a loss of the ß-haemolytic phenotype on blood agar plates and non-haemolytic mutants were selected for further investigation. By sequencing the insertion sites of these mutants we identified thus far 10 different mutations sites in a gene cluster of 9 kb harbouring 9 open reading frames, with significant similarities to the *sag* (SLS associated gene) gene cluster of *Streptococcus pyogenes* that encodes the haemolysin Streptolysin S (SLS). ORFs corresponding to all of the 9 *sag* genes (*sagA* to *sagI*) could be identified. Similarities of the deduced amino acids of the putative *S. anginosus sag* gene cluster to the Sag-proteins of *S. pyogenes* range from 37 % (sagF) to 81 % (sagD). To further investigate the *S. anginosus* haemolysin, a functional haemolysin assay with culture supernatants and whole bacteria was carried out. Haemolytic activity was only observed with whole cells, not in the supernatant, indicating that like SLS of *S. pyogenes*, the *S. anginosus* haemolysin is able to lyse erythrocytes only in cell-associated form. But contrary to *S. pyogenes*, adding of FCS to the cell-free supernatant of *S. anginosus* did not increase haemolytic activity.
In summary we were able to identify an SLS-like gene cluster as the genetic basis of *S. anginosus* ß-haemolysin production and could further characterize the ß-haemolysin.

MPV6-FG

Alternative pathways of phagosomal escape of *Staphylococcus aureus*

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Staphylococcus aureus is efficiently taken up by non-professional phagocytes. Subsequently, certain bacterial strains are able to escape the phagoendosome in an *agr*-controlled process. We have recently identified that expression of *S. aureus* δ-toxin, an *agr*-effector encoded by *RNAIII*, can augment phagosomal escape in presence of β-toxin. Here we show that expression of phenol soluble modulin (PSM) β but not PSMα also leads to escape of β-toxin positive S. aureus. By contrast, the membrane-destructive activity of PSMα is supported by another phospholipase. Thus, alternative pathways exist for phagosomal escape of *S. aureus*, which might present different strategies to avoid lysosomal disinfection in presence or absence of β-converting phages.

MPP1-FG

Characterization of a novel genomic island in a monophasic variant of *Salmonella* Typhimurium

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Besides the core genome, bacterial chromosomes harbour numerous accessory genes acquired by horizontal gene transfer. Organised in clusters predominantly at tRNA loci, these genes are recognised as genomic islands (GEIs). Depending on their gene content the elements are termed pathogenicity, symbiosis, metabolic, fitness or resistance islands [1]. Here we describe a novel 18.4 kb genomic island adjacent to the *thrW*tRNA locus of an endemic monophasic variant of *Salmonella* Typhimurium. 454 sequencing and subsequent BLAST analyses revealed 27 open reading frames and a significantly lower G+C content compared to the closely related *S.* Typhimurium LT2 genome. Homologies covering large parts of the island have been found to several *E. coli* and *Shigella* nucleotide sequences [2]. Protein BLAST analyses revealed a number of phage-related proteins, indicating that the island might be of phage-origin. Transcripts were detected for 24 ORFs. Further we showed that the island can be excised from the chromosome and form a circular intermediate which is mobilised under certain conditions. Broth mating experiments resulted in the successful conjugational transfer of the 18.4 kb island from the donor to an appropriate *S.* Typhimurium recipient strain. To elucidate the function of the island, we focussed on ORF 10 since it is predicted to code for a T3SS effector. Our experiments suggest that its product is truly secreted but probably not via the SPI1-, SPI2- or flagella-T3SS described

forS. Typhimurium. Uncovering the function of the ORF 10 gene product and the identification of potential interaction partners would provide essential information to understand the relevance of the whole island in the emerging monophasic variant of *Salmonella* Typhimurium.

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MPP2-FG

Important codon positions and unusual anomalies in microbial 16S RNA sequences

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In most microbial RNA seqeunces there are particular regions of the sequence that show a priority for important translations especially when the organism is producing specific substances for its own survival mechanisms and for incorporation and use in both intercellular and intracellular activities. These codon sequences are needed for the production of polysaccharides which are used both inside and outside the cell wall so are both exopolysaccharides and intrapolysaccharides. However these particular codon sequences are not always as regular as expected and have some unusual anomalies especially with the advent of AAA and AAAA repetitions. These may not seem unusual at first but their importance becomes apparent with the gradual production of the intra and extracellular products. The two species that will be considered that provide such unusual sequences are firstly xanthomonas, a plant pathogen and secondly clostridia, a human pathogen. The important codon sequences and anomalies for these species will be considered.

MPP3-FG

Complete fiber structure of the trimeric autotransporter adhesin SadA

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Trimeric autotranporter adhesins (TAAs) represent a group of non-fimbrial, non-pilus adhesins that are widespread in α-, β-, and g-proteobacteria. They include a number of prominent pathogenicity factors including *Yersinia* YadA, *Neisseria* NadA and *Bartonella* BadA that are involved in pathogen adhesion as well as in the defence against host responses. TAAs are targeted by the type Vc secretion pathway through the outer membrane into the extracellular space. Their architecture follows a general head-stalk-anchor assembly from the N- to the C-terminus. TAAs are highly modular multidomain proteins with a variable number of head and stalk domains that are linked by several types of connector domains. The highly conserved C-terminal membrane anchor harbours the autotransporter function and defines the protein family [1].
In order to explore the domain diversity of trimeric autotransporter adhesins, we set out to produce a dictionary approach (daTAA, available at http://toolkit.tuebingen.mpg.de/dataa) which allows the detailed and automated annotation of TAAs from sequence data [2]. daTAA provides information on the sequence, structure and function of so far 25 different domain types as well as the rules by which these are combined to form the observed long fibers on the cell surface.
As complete TAA fibers are not amenable for X-ray crystallography, we turned to solve the structures of single domains in order to assemble them into the full fiber *in silico* in a later step. The *Salmonella* adhesin SadA served as a perfect model as it is a highly complex adhesin composed of different types of domains. Closest SadA homologues are found in almost all enterobacteria, such as UpaG, an adhesin involved in the infection process of uropathogenic *E. coli*. Exploiting the observation that almost all domain types of TAAs begin and end in coiled-coil segments, we produced a pASK IBA - based expression vector system that fuses the extremely stable trimeric pII variant of the GCN4 leucine zipper in register to the N- and C-terminal ends of the domain constructs [3, 4]. We solved the structure of all exemplars of domain types of SadA by molecular replacement and assembled them together with homology models of isolated domains into a complete structural model of the full SadA fiber. Our work successfully approved the applicability of the dictionary approach to understand the structural organization and to perform the annotation of this complex class of proteins.

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OTV001

The first structure of a LanI protein, SpaI: The protein conferring autoimmunity against the lantibiotic subtilin in *Bacillus subtilis* reveals a novel fold

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The careless use of many antibiotics in the past lead to emerging resistances even against 'last resort' drugs such as vancomycin. Thus, there is an urgent need for structurally novel antimicrobial agents. Lantibiotics are small ribosomally synthesized peptide antibiotics with posttranslational modified amino acids resulting in the characteristic lanthionine and methyllanthionine bridges.
Bacillus subtilis ATCC 6633 produces the lantibiotic subtilin which damages the cell wall of gram-positive bacteria. SpaI is a 16.8 kDa lipoprotein which is part of the self-protection system of *B. subtilis* against subtilin. It is attached to the outside of the cytoplasmic membrane *via* a covalent diacylglycerol anchor. SpaI together with the ABC-transporter SpaFEG protects the membrane from subtilin insertion.
We solved the structure of a 15 kDa biologically active fragment of SpaI by NMR which is the first structure of any LanI (lanthionine immunity) protein from lantibiotic producing strains. A search in the DALI database indicated a novel fold for SpaI. Our data show that SpaI has as mainly β-strand structure with seven β-strands and two α-helices[1]. NMR investigations of a full length construct of SpaI lacking the diacylglycerol anchor suggest that the 30 N-terminal amino acids are unfolded in the absence of a membrane. However, this N-terminal stretch shows interactions with liposomes in NMR titration experiments. When mutating this stretch *in vivo* the SpaI mediated immunity of *B. subtilis* against subtilin is not affected and lipobox mutants of SpaI are still found in the membrane fraction.
Our results are the first step on the way to understand subtilin autoimmunity of *B. subtilis* on a structural level at atomic resolution.

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OTV002

Analysis of SpaI-mediated lantibiotic immunity in *Bacillus subtilis*

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Lantibiotics are lanthionine-containing peptides [1] that exhibit antimicrobial as well as pheromone-like autoinducing activity [2]. *Bacillus subtilis* ATCC 6633 produces the cationic pore-forming lantibiotic subtilin, which acts on Gram-positive microorganisms by interfering with the lipid II cycle essential for peptidoglycan biosynthesis [3]. Self protection of the producer cells is mediated by the lipoprotein SpaI and the SpaFEG ABC-transporter [4]. SpaI as typical lipoprotein is anchored to the outer membrane via a diacylglycerol moiety.
Different SpaI mutations were generated to elucidate the mechanism of SpaI-mediated immunity. In contrast to other membrane bound lipoproteins, replacement of the cysteine within the lipobox-motif "LSAC" by alanine did not release the protein from the membrane. This result indicates that the membrane interaction of the mature protein occurs also in the absence of lipid-modification. Based on structural elucidation, two domains (domain 1 and domain 2) were identified, which are indispensable for SpaI function. Surprisingly, if amino acid residues of domain 1 were newly aligned, the mutated $SpaI^{D1mix}$ protein was still functional. The current data suggest that the overall charge of domain 1 is decisive for its function, and not its primary sequence. Domain 2 is also indispensable for SpaI function and needs to be entirely conserved.
Our current data suggest that the N-terminal domain of SpaI is important for membrane association in addition to the diacylglycerol anchor.

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OTV003

First crenarchaeal chitinase detected in *Sulfolobus tokodaii*

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Chitin is after cellulose the second most abundant biopolymer on earth, consisting of beta 1,4-glycosidic bonded N-acetyl-glucosamine subunits with various grades of acetylation. It is wide spread from deserts to the deep sea, generated mostly by arthropoda and fungi with a production and steady state amount of an estimated 10^{10} to 10^{11} tons per year [1]. Chitin degradation is an extremely important step in nutrient cycling especially in the oceans [2] and comprises the combined action of several enzymes. During the degradation process chitinases (EC3.2.4.14) mainly hydrolyse the beta 1,4-glycosidic bonds within the chitin polymer. Although chitinases are widely distributed in all domains of life, only little is known about archaeal chitinases. Within the domain of archaea, only ten euryarchaeal chitinases were found so far in terms of genetic or molecular information. Until now, no chitinases or chitinase genes were described or annotated from crenarchaea.

Here we show that the ORF BAB65950 from *Sulfolobus tokodaii* str. 7 encodes for the first functional crenarchaeal chitinase. The ORF was expressed in *E. coli* and the resulting protein degraded chitin. It was hence classified as a chitinase (EC 3.2.4.14). The protein characterisation revealed a specific activity of 75 mU/mg when incubated with colloidal chitin as substrate. The optimal activity of the enzyme was at pH 2.5 and 70°C. A dimeric enzyme configuration is proposed. The derived amino acid sequence of the enzyme could neither be attributed to the glycoside hydrolase family 18 nor 19. However, within a phylogenetic sequence tree, the deduced amino acid sequence of the ORF clustered into close proximity of members of the glycoside hydrolase family 18 [3].

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OTV004

A novel biosynthetic pathway for the synthesis of Archaea-type ether lipids in Bacteria

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The universal tree of life divides all organisms into the phylogenetic superkingdoms Eukarya, Bacteria and Archaea, which differ by the chemical composition of their membrane lipids. Lipids from Bacteria and Eukarya are composed of a *sn*-glycerol-3-phosphate core to which fatty acids are bound via ester linkages, while lipids from Archaea consist of *sn*-glycerol-1-phosphate (G1P) to which polyprenyl chains are attached by ether bonds.

This difference has suggested that the emergence of the Archaea during evolution was linked to the advent of glycerol-1-phosphate dehydrogenase (G1PDH) and geranylgeranylglyceryl phosphate synthase (GGGPS). These enzymes catalyze the first two steps leading to G1P-based ether lipids, the reduction of dihydroxyacetone phosphate to G1P, and the condensation of G1P with geranylgeranyl pyrophosphate to geranylgeranylglyceryl phosphate.

We were interested to elucidate the function of the hitherto uncharacterized AraM and PcrB proteins, which show a significant sequence similarity to the archaeal G1PDH and GGGPS, respectively, but occur in gram-positive bacteria such as *Bacillus subtilis*. We first showed that AraM is a Ni^{2+}-dependent G1PDH [1]. We then analyzed the function of PcrB, which is a homologue of the archaeal GGGPS and therefore was assumed to link G1P generated by AraM with an unknown polyprenyl pyrophosphate substrate, yielding a specific ether lipid. We developed a protocol for the identification of this substrate of PcrB which is based on its reaction with ^{14}C-G1P and the subsequent isolation of the formed radio-labeled ether lipid product from *B. subtilis* cells. The results showed that PcrB catalyzes the reaction of G1P with heptaprenyl pyrophosphate to heptaprenylglyceryl phosphate, which is subsequently dephosphorylated and acetylated.

The functional assignment of AraM and PcrB has allowed us to identify a hitherto unknown pathway for the biosynthesis of archaea-type ether lipids in gram-positive bacteria. Moreover, we show that the different substrate specificities of the archaeal GGGPS and the bacterial PcrB, which bind polyprenyl moieties containing 20 and 35 carbon atoms, respectively, are caused by a single amino acid difference at the bottom of the active site [2].

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OTV005

De novo structure of the membrane anchor domain of the trimeric autotransporter YadA by solid-state NMR spectroscopy

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Question: Solid-state magic-angle spinning (MAS) NMR spectroscopy has long been discussed as the emerging method of choice for membrane protein structural biology (1,2). MAS NMR does not necessarily need highly and macroscopically ordered material and is not hampered by slow tumbling. Moreover, solid-state NMR is a unique tool to study both dynamics and structure of proteins simultaneously at atomic resolution (3-5). YadA is a trimeric autotransporter adhesin (TAA (6)). Many members of the TAA family are important pathogenicity factors that mediate adhesion to host cells and tissues in such diverse diseases as diarrhea, urinary tract infections, or airway infections. The common structural features of TAAs are trimeric domains with a high content of alpha-helical coiled coils and of beta-helical or beta-trefoil structures (6). These domains occur in varying order and repeat number in different bacterial TAAs, but the defining element of the family is the membrane anchor (or translocator) domain which hosts two important functions. It anchors the adhesin in the bacterial outer membrane and exports all other, extracellular domains to the cell surface - hence the name, autotransporter. The mechanism of this autotransport is poorly understood.

Methods: solid-state magic angle spinning NMR

Results: Here, we present the first structure of a membrane protein, the transmembrane domain of the Yersinia Adhesin A (YadA), solved exclusively with solid-state MAS NMR data, using a single, uniformly 13C/15N labeled sample.

Conlusions: The first partial structure of a TAA was obtained for the collagen-binding, extracellular head domain of YadA from the enteropathogenYersinia enterocolitica (7). Thus far, only for one TAA anchor domain, of Haemophilus Hia, an x-ray structure has been obtained (11). We applied solid-state MAS NMR to crystalline YadA-M to collect high-resolution structural data. In addition, NMR allowed us to acquire information on flexibility and other mechanistic detail that cannot be transferred from the x-ray structure of Hia (11).

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OTV006

Biochemical and structural analysis of FlaH, a component of the crenarchaeal flagellum

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Motility is a very important attribute of live. It allows the organisms from all three domains of life to adapt to a changing environment, which is crucial for the survival of various species. The two most commonly used motility structures in bacteria are flagella and type IV pili. Flagella are involved in swimming motility whereas type IV pili are mostly involved in twitching motility. Both modes of movements have been extensively studied and the assembly systems and functions are well characterized. This is not the case for archaeal motility. Numerous cell appendages such as flagella and pili have been already identified, but still not much is known about their assembly mechanisms and functions. We are mostly interested in the archaeal flagellum, which is a unique motility apparatus that performs the same function as bacterial flagella; although it is structurally more related to bacterial type IV pili. In most known flagellated archaea the flagella-associated genes are organized in a single *fla* gene cluster, consisting of flagellin encoding genes (*flaA, flaB*), some variations of genes encoding accessory proteins (*flaCDEGFH*), genes encoding an ATPase (*flaI*) and a polytopic membrane protein (*flaJ*).

Using *Sulfolobus acidocaldarius* as a model organism we want to analyze the crenarchaeal flagella assembly system and its function. We could already show that all seven genes encoded in the *fla* gene cluster of *Sulfolobus acidocaldarius* are essential for crenarchaeal flagella assembly and for swimming motility in liquid environments. My project is mainly focused on the *in vivo* and *in vitro* study of *S. acidocaldarius* flagella component FlaH. FlaH is an incomplete ATPase, which contains a well defined Walker A, but lacks the Walker B motive. As we determined the structure of FlaH and using this, we constructed defined point mutants. Combining mutant analysis with biochemical studies will help us to

understand the exact role of FlaH in the assembly and function of the crenarchaeal flagellum.

OTV007

Subcellular positioning of a DNA-binding protein through constraint movement

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Many protein complexes localize to defined regions within cells. The bacterial SMC complex consists of a central SMC dimer and two accessory factors, ScpA and ScpB. SMC binds non-specifically to DNA *in vitro*, while ScpA and ScpB appear to confer a regulatory function. The complex plays an important role in chromosome condensation and segregation during the bacterial cell cycle, and forms two discrete subcellular centres, one in each cell half, when imaged with conventional epi-fluorescence microscopy. Using single molecule microscopy and tracking we show that localization is achieved through limited yet rapid movement of the SMC subunits through a cell half, while the accessory ScpAB subunits mediate temporal arrest of a subset of SMC molecules at the centre of a cell half. Thus, specific localization is achieved by movement through the nucleoid and transient arrest at the nucleoid centre. FRAP studies show that the SMC pool has a high turnover within a cell half and is also replenished through *de novo* protein synthesis, yielding an additional level of protein dynamics. Diffusion/movement within a limited compartment and transient arrest may be a general means to accumulate proteins within non-compartmentalized cells.

OTV008

Protein complexes involved in the electron transport chain of anammox bacteria

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Anammox bacteria combine ammonia with nitrite to dinitrogen gas with nitric oxide and hydrazine as intermediates (1). Oxidation of the latter yields low-redox-potential electrons, which can be used for CO_2 fixation. We hypothesize that these are replenished through the oxidation of nitrite to nitrate by a nitrite oxidizing system (NAR) (2). As nitrite is a relatively poor reductant, the electrons have to be energized to enter the bc_1-complex or to feed a quinone pool, which implies reverse electron transport.
The gene cluster that contains the catalytic subunits of nitrite oxidizing (narGH) system covers almost the full natural repertoire of electron carriers. This includes genes encoding six putative heme-containing proteins and two putative blue-copper proteins and a putative anchor to the membrane showing homology to a cytochrome bd oxidase subunit (2).
Furthermore, the genome of the anammox bacterium *Candidatus* Kuenenia stuttgartiensis shows a high redundancy of respiratory genes, suggesting an intricate cellular electron transport system. Interestingly, the three operons encoding for the bc_1 complexes, complex III in the respiratory chain, all differ in their subunit composition from the canonical bc_1 complexes in other microorganisms. One operon consists only of a heme b /c fusion protein and the Rieske protein. The other two operons encode for multi heme c containing genes, NAD(P) oxidoreductase subunits and, intriguingly one of them contains a hydroxylamine oxidoreductase subunit. The combination of these subunits strongly suggests that electrons derived from different oxidation reactions could be wired to different electron acceptors, once entering the bc_1 complexes.
The whole protein complement of *K. stuttgartiensis* membranes was determined with protein correlation profiling using LC-MS/MS data from consecutive Blue Native (BN) gel slices (3). The detection of different complexes was coupled to in-gel activities of the respiratory complexes in BN gels. Further, the catalytic subunit of the nitrite oxidizing system of *K. stuttgartiensis* was purified.

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OTV009

Replication fork movement and methylation governs SeqA binding to the *Escherichia coli* chromosome

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Chromosomes are composed of enormously long DNA molecules which must be distributed correctly as the cells grow and divide. In *Escherichia coli* the SeqA protein might be involved in organization of new DNA behind the replication forks. SeqA binds specific to GATC sequences which are methylated on the A of the old strand but not on the new strand. Such hemi-methylated DNA is produced by progression of the replication forks and lasts until Dam methyltransferase methylates the new strand. It is therefore believed that a region of hemi-methylated DNA covered by SeqA follows the replication fork. We show that this is indeed the case by using global ChIP on Chip analysis of SeqA in cells synchronized regarding DNA replication. To assess hemi-methylation we developed the first genome wide method for methylation analysis in bacteria. A comparison of rapid and slow growth conditions showed that in cells with multiple replication forks per chromosome, the old forks bind little SeqA. Analysis of strains with strong SeqA binding sites at different chromosomal loci supported this finding. The results indicate that a re-organization of the chromosome occurs at a timepoint coinciding with the end of SeqA dependent origin sequestration. We suggest that a reorganization event occurs resulting in both origin desequestration and loss of old replication forks from the SeqA structures.

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OTV010

Translocation of sodium ions by the ND5 subunit of mitochondrial complex I from the yeast *Yarrowia lipolytica*

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Mitochondrial complex I (NADH:ubiquinone oxidoreductase), localized in the inner mitochondrial membrane, is the first enzyme of the electron transport chain of the oxidative phosphorylation system. The L-shaped complex is partitioned into a peripheral arm and a membrane-bound domain.
In the peripheral arm electrons from NADH are transferred to ubiquinone via iron sulfur clusters, using FMN as cofactor. This process is coupled, by conformational changes as structural data indicates, with the translocation of protons by the membrane-bound domain (Brandt 2006; Efremov, Baradaran et al. 2010; Efremov and Sazanov 2011). Here we focus on the ND5 subunit of the membrane-bound domain of the mammalian complex which is considered to be involved in the translocation of protons. Previous studies showed that the ND5 homologue NuoL from *E. coli* complex I transports sodium ions across the membrane (Gemperli, Schaffitzel et al. 2007). We also observed that ND5 from human complex I, when inserted into the inner mitochondrial membrane of *S. cerevisiae*, leads to an increased salt sensitivity of the yeast cells, suggesting that ND5 promotes the leakage of cations across the mitochondrial membrane (Steffen, Gemperli et al. 2010). Here, we investigate the cation transport activity of the ND5 homologue from the yeast *Y. lipolytica* produced as GFP-ND5 fusion protein in ER vesicles from *S. cerevisiae*. The topology of ND5 in the vesicles was analyzed by limited proteolysis. The N-terminal GFP fusion to ND5 was oriented towards the external lumen of ER vesicles. This uniform orientation of ND5 in vesicles was the prerequisite for cation transport studies where a Na^+ concentration gradient was applied. ER vesicles containing GFP-ND5 exhibited a significantly higher Na^+ uptake activity than control vesicles without ND5. This indicates that the individual ND5 protein which is highly related to secondary Na^+/H^+ antiporters contains a channel for Na^+.

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OTV011

Large and frequent introns in the 16S rRNA genes of large sulfur bacteria

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The gene encoding the small ribosomal subunit (16S/18S rDNA) serves as a prominent tool for the phylogenetic analysis and classification of living organisms owing to its high degree of conservation and its fundamental function[1]. Nowadays, established methods to analyze this gene are taking advantage of its conservation in size and nucleotide composition[2]. We sequenced the 16S rRNA genes of not yet cultivated large sulfur bacteria, among them the largest known bacteriumThiomargarita namibiensis, and found that the genes regularly contain numerous self-splicing introns of variable length. The 16S rRNA genes of these bacteria can thus be enlarged to up to 3.5 kb.
Using a modified CARD-FISH approach we can show that the introns are transcribed as part of the rRNA precursor, but they cannot be located in the native ribosomes. Also, the introns show self-splicing abilities in in vitroexperiments, i.e. they autonomously excise from RNA and mediate the ligation of the two exons. These findings lead to the conclusion that the introns are capable of independent removal during ribosome maturation, therefore minimizing negative impact on the host organism. Remarkably, introns have never been identified in bacterial 16S rRNA genes before, although being the most frequently sequenced gene today. This may be caused in part by a bias during the PCR amplification step discriminating against longer homologues, as we can show experimentally as well. The fact that introns were now located in the 16S rRNA genes in the large sulfur bacteria, and have also been found in the 23S rRNA genes of several other bacteria[3,4], implies that the presence of introns in the bacterial rRNA operon is more common than previously recognized. Possibly, also other groups of bacteria likewise have introns in their 16S rRNA genes, which would have profound implications for common methods in molecular ecology - it may cause systematic biases and lead to the exclusion of the intron-containing fraction of a heterogeneous population. The general impact of this finding on the standard analysis of rRNA genes is apparent.

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[5] This study was funded by the Max Planck Society.

OTV012

Regulation of anaerobic respiratory pathways in *Dinoroseobacter shibae*

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Denitrification is part of the global nitrogen cycle and an important mechanism of energy generation under anaerobic conditions. *Dinoroseobacter shibae*, a representative of the globally abundant marine *Roseobacter* clade, is used as a model organism to study the transcriptional response to changing oxygen conditions in the presence of nitrate. Its annotated 4.4 Mb genome sequence revealed clustered genes, which are involved in anaerobic respiratory energy metabolism with nitrate as alternative electron acceptor [1]. Interestingly, *D. shibae* contains the periplasmic nitrate reductase Nap instead of the membrane bound Nar. *D. shibae* features *nir*, *nor* and *nos* operons in the vicinity of the *nap* operon. An unusual high number of Crp/Fnr-like regulators have been predicted: Beside one FnrL-homologue with a $[4Fe\text{-}4S]^{2+}$-cluster, six Dnr-like regulators are found. The genes encoding DnrD and DnrE are directly located between the *nor*- and *nos*-operon. We are interested in identifying gene regulatory patterns after shifting from aerobic to anaerobic denitrifying conditions. Therefore, we used continuous cultivation of *D. shibae* in a chemostat combined with time series microarray analysis. We detected anaerobic growth of *D. shibae* via denitrification. Transcriptome analysis revealed distinct patterns of gene expression in response to oxygen limitation. The change from aerobic to anaerobic growth showed a sequential induction of gene clusters encoding the four reductases of the denitrification machinery. Genes encoding Fnr/Crp-like regulators showed different expression levels over time. In response to oxygen limitation, an immediate upregulation of universal stress proteins, fine-tuning of the electron transport chain components, as well as the downregulation of the translational apparatus was observed. Furthermore, we predict a regulatory network for the anaerobic respiratory pathway in *D. shibae*.

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OTV013

Influence of subcellular antigen localization within different yeast genera on the activation of ovalbumin-specific CD8 T lymphocytes

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Yeasts of the genus *Saccharomyces* expressing recombinant antigens are currently evaluated as candidate T cell vaccines. We compared the interaction kinetics between four biotechnologically relevant yeast genera (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis* and *Pichia pastoris*) and human dendritic cells. Further, we analyzed the activation capacity of recombinant yeasts expressing ovalbumin (OVA) either intracellular, extracellular or surface-displayed by OVA-specific CD8 T lymphocytes. We found that the kinetic patterns of yeast uptake by phagocytic cells varied between the tested yeast genera and that both genus and subcellular OVA antigen localization influenced the strength of T cell activation. In particular, in *S. cerevisiae*, a secreted antigen was less effectively delivered than its cytosolic variant, whereas most efficient antigen delivery with *P. pastoris* was obtained by cell surface bound antigen. Our data indicate that protein secretion might not be an effective delivery pathway in yeast. [Bazan et al. (2011) Vaccine 29; 8165]

OTV014

The quest for new oxidative catalysts: Expression of metagenomic membrane-bound dehydrogenases from acetic acid bacteria in *Gluconobacter oxydans*

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Acetic acid bacteria are used in biotechnology due to their ability to incompletely oxidize a great variety of carbohydrates, alcohols and related compounds. Many of these oxidations are unfeasible using organic chemistry. Because these reactions are mostly catalyzed by membrane-bound dehydrogenases, in a rapid, regio- and stereo-selective manner, the substrates do not have to be transported into the cytoplasm. Due to the fact that many acetic acid bacteria can not be cultivated in the laboratory we use a metagenomic approach to indentify new membrane-bound dehydrogenases of potential value for biotechnology from a mother of vinegar.
The membrane-bound dehydrogenases are screened by sequence similarity from the metagenomic library and are functionally expressed in specially designed *Gluconobacter oxydans* strains. In these strains all membrane-bound dehydrogenases were deleted using a clean deletion system developed by our group to avoid overlapping enzymatic specificities. Using specifically designed expression vectors we ensure functional integration in the membrane physiology of these organisms.
In order to set up a high throughput assay to characterize the activity of membrane-bound dehydrogenases, we developed a whole cell system in microtiter-plates. The advantage of this system is a minimized cell preparation together with the ability to compare many stains or substrates in one experiment. We used this approach to determine the *in vivo* substrate spectrum of several membrane-bound dehydrogenases from acetic acid bacteria for the first time.

OTV015

Growth phase dependent changes of the RNA degrading exosome in Sulfolobus solfataricus

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We are investigating the exosome of the hyperthermophilic and acidophilic archaeonSulfolobus solfataricus(1).The archaeal exosome is a protein complex involved in the degradation and the posttranscriptional tailing of RNA. The core of the complex is build of a phosphorolytically active hexameric ring of the subunits Rrp41 and Rrp42, to which a trimeric cap of the RNA-binding proteins Rrp4 and/or Csl4 attaches (2). Rrp4 and Csl4 confer different substrate specificity to the exosome (3). In addition to these subunits, the archaeal DnaG protein is stably associated with the exosome (4). The majority of the protein complex including DnaG is localized at the periphery of the cell and is detectable in the non-soluble fraction (5). Here we show that DnaG directly interacts with Csl4 in the exosome, and that it differently influences the activity of complexes with homotrimeric Rrp4- or Csl4-capsin vitro. We confirmed the existence of

heterotrimeric, Rrp4- and Csl4-containing capsin vivo. Furthermore, we observed increased amounts of soluble, Rrp4-containing exosome in the stationary phase, when the vast majority of the DnaG-Csl4-exosome remains non-soluble. Our data strongly suggest that temporal and spatial changes in the localization of the exosome are based on changes in the composition of the RNA-binding cap and its interaction with DnaG.

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OTV016

Freshwater Actinobacteria acI as revealed by single-cell genomics

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Actinobacteria of the acI clade are often numerically dominating freshwater ecosystems where they can contribute >50% of the bacteria in the surface water. However and as often with environmentally important species they are uncultured to date. That is why we set out to study their genomic information in order to learn about their physiology and ecological niche. We used a single cell genomics approach which consisted of the following steps: (1) single cell sorting by Fluorescence-activated cell sorting (FACS), (2) whole genome amplification (WGA) using Phi29 DNA polymerase, (3) screening of SAG (Single cell amplified genome) DNA by 16S rRNA sequencing, (4) shotgun genomic sequencing followed by (5) genome assembly, annotation and data analysis using The Joint Genome Institute's (JGI) Integrated Microbial Genomes (IMG) analysis platform. We obtained a draft genomic sequence in 75 larger contigs (sum = 1.16 Mbp) and with an unusual low genomic G+C mol% (i.e. ~42%). Single copy gene analysis suggests an almost complete genome recovery. We also noticed a rather low percentage of genes with no predicted functions (i.e. ~15%) as compared to other cultured and genome-sequenced microbial species. Our metabolic reconstruction hints at the degradation of pentoses (e.g. xylose) instead of hexoses. We also found an actinorhodopsin gene that may contribute to energy conservation under unfavorable conditions. This project reveals the possibilities and limitations of single cell genomics for microbial species that defy cultivation to date.

OTV017

Carbon and hydrogen isotope fractionation during nitrite-dependent anaerobic methane oxidation by *Methylomirabilis oxyfera*

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Anaerobic oxidation of methane coupled to nitrite reduction is a recently discovered methane sink of as yet unknown global significance. The bacteria that have been identified to carry out this process, Candidatus *Methylomirabilis oxyfera*, oxidize methane via the known aerobic pathway involving the monooxygenase reaction [1]. In contrast to aerobic methanotrophs, oxygen is produced intracellularly and used for the activation of methane by a phylogenetically distinct particulate methane monooxygenase (pMMO) [1]. Here we report the fractionation factors for carbon and hydrogen during methane degradation by an enrichment culture of *M. oxyfera* bacteria. In two separate batch incubation experiments with different absolute biomass and methane contents, the specific methanotrophic activity was similar and the progressive isotope enrichment identical. The enrichment factors determined by Rayleigh approach were in the upper range of values reported so far for aerobic methanotrophs. In addition, two-dimensional specific isotope analysis ($\Lambda = (\alpha_H^{-1}-1)/(\alpha_C^{-1}-1)$) was performed and also the determined Λ value was within the range determined for other aerobic and anaerobic methanotrophs. The results showed that in contrast to abiotic processes biological methane oxidation exhibits a narrow range of fractionation factors for carbon and hydrogen irrespective of the underlying biochemical mechanisms. In contrast to aerobic proteobacterial methanotrophs, *M. oxyfera* does not assimilate its cell carbon from methane. Instead, only the Calvin-Benson-Bassham cycle of autotrophic carbon dioxide fixation was shown to be complete in the genome, as well as transcribed and expressed [2]. Further experiments are conducted in order to experimentally validate the proposed incorporation of carbon dioxide into cell biomass.

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OTV018

Characterization of Novel Bacterial Alcohol Dehydrogenases Capable of Oxydizing 1,3-propanediol

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1,3-propanediole (1,3-PD) is a valuable compound for textile fiber, film and plastic industry. It is chemically produced from acrolein or ethylene oxide via 3-hydroxypropionaldehyde (3-HPA). Since the chemical production of 1,3-PD is expensive and goes along with the formation of toxic side products, much effort has been taken to establish a microbiological production system. Facultative anaerobic microorganisms have been investigated with regard to their capability to produce 1,3-PD from glycerol. In a 2-step reaction, glycerol is converted to 3-HPA and the latter is finally reduced to 1,3-PD by a 1,3-propanediol oxidoreductase (PDOR). The second reaction has been shown to be catalyzed by non-specific alcohol dehydrogenases (ADH) as well. Since only a few PDOR have been investigated in detail, an approach to identify and characterize ADH with novel properties for the production of 1,3-PD has been established. BLAST searches were performed using the sequences of PDOR and related ADH with known activity towards 3-HPA or 1,3-PD from species of the genera *Citrobacter*, *Clostridium*, *Klebsiella*, and *Escherichia coli*. Putative homologues were identified in the genome of the bacterial species *Oenococcus oeni*, *Dickeya zeae*, *Pectobacterium atrosepticum*, *Pelobacter carbinolicus* and from sequenced metagenomes derived from uncultivated bacteria living in deep sea-sediments. A total of 10 different open reading frames were cloned into pQE30 expression vectors and were purified after heterologous production in *E. coli*. Results on the evolutionary relationships and biochemical properties of the enzymes will be presented.

OTV019

Bacterial CYP153 monooxygenases as biocatalysts for the synthesis of ω-hydroxy fatty acids

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ω-Hydroxy fatty acids (ω-OHFAs) and α,ω-dicarboxylic acids (α,ω-DCAs) are multifunctional compounds useful for the production of polymers, lubricants, cosmetics and pharmaceuticals. Recently, medium- to long-chain saturated ω-OHFAs have attracted considerable attention for their use as precursors of poly(ω-hydroxy fatty acids) [1]. These polymers exhibit similar or even superior physicochemical properties compared to polyethylene and other bioplastics. Long-chain *cis*-monounsaturated ω-OHFAs and α,ω-DCAs are also valuable because they yield polymers that can be cross-linked or chemically modified at their double bond sites [2].
Cytochrome P450 monooxygenases (CYPs) are enzymes that use molecular oxygen to insert one oxygen atom into non-activated hydrocarbons. During the last two decades several eukaryotic CYPs have been isolated and engineered for the yeast-based production of ω-OHFAs and α,ω-DCAs [3]. Bacterial CYP153A enzymes are soluble alkane ω-hydroxylases [4] whose activity towards fatty acids has not been reported yet. As certain CYP153A convert primary alcohols to α,ω-diols [5,6], we presumed they ω-hydroxylated fatty acids as well.
We functionally expressed CYP153A from *Polaromonas* sp., *Mycobacterium marinum* and *Marinobacter aquaeolei* in *E. coli* to investigate their *in vitro* fatty acid oxidation profiles. Here we demonstrate for the first time that CYP153A enzymes oxidize fatty acids to ω-OHFAs and, sometimes, further to α,ω-DCAs. CYP153A from *M. aquaeolei* was identified as a fatty acid ω-hydroxylase with a broad substrate range. This biocatalyst produced ω-OHFAs from medium-chain saturated and long-chain *cis*/*trans*-monounsaturated fatty acids with 64 - 93% conversion and >95% ω-regioselectivity. Our study gives further insight into the physiology of ω-oxidizing bacteria and provides the basis for the development of a recombinant *E. coli* system to synthesize ω-OHFAs from renewable feedstocks.
We acknowledge financial support from the German Federal Ministry of Education and Research (BMBF) in the frame of the "Systems Biology in *Pseudomonas* for Industrial Biocatalysis" project as well as the European

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OTV020

Using yeast and fungi to produce electricity - Towards a self-regenerating enzymatic biofuel cell cathode

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Biofuel cells (BFCs) directly transform chemical energy into electricity for as long as fuel and oxidant are supplied. To catalyze the electrode reaction in biofuel cells, for instance biochemical pathways of complete microorganisms or enzymatic biocatalysts can be used [1].
The aim of our research is to improve the long-term stability of efficient, but currently short-lived enzymatic biofuel cell electrodes [2]. We aim to continually supply catalytically-active enzymes at the electrode using living microorganisms that grow in an electrode-integrated micro-bioreactor.
In the present work, we demonstrate the feasibility of using the crude culture supernatant of the fungus *Trametes versicolor* and the recombinant yeast *Yarrowia lipolytica* [3] to supply the biocatalyst laccase to a biofuel cell cathode. Both *T. versicolor* and *Y. lipolytica* were grown in a synthetic deficient (SD) medium. At approximately the highest enzyme activity, which was 3.6 U/ml for *T. versicolor* and 0.02 U/ml for *Y. lipolytica*, culture supernatant was transferred into a biofuel cell cathode compartment [4]. To record the loadcurve, current was incrementally increased (steps of 5.6 μA/(cm^2*h)) and the cathode potential was measured against a saturated calomel electrode (SCE). At a cathode potential of 0.4 V vs. SCE, we obtained a current density of 134 μA/cm^2 for *T. versicolor*. The same enzyme activity of commercial *T. versicolor* laccase (Sigma) in SD medium yielded a current density of only 75 μA/cm^2 and in citrate buffer a current density of 87 μA/cm^2. For *Y. lipolytica*, a current density of 4 μA/cm^2 was measured. The same amount of purified laccase from *Y. lipolytica* in SD medium and in citrate buffer resulted in a current density of 8 μA/cm^2 and 12 μA/cm^2 respectively.
Our results are a first step towards constructing a self-regenerating enzymatic biofuel cell with extended lifetime. Furthermore, we have shown that the choice of microorganism, has an influence on the obtained current density, because it has a large influence on the composition of the culture supernatant as well as on laccase activity. Important topics for future work will be the clarification of the secreted byproducts and the integration of the laccase-producing microorganisms in the electrode compartment.
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OTV021

Biofilms - a new Chapter in Biocatalysis

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In biocatalysis, the traditional bottlenecks like low biocatalyst stability, toxicity problems, and difficulties in running continuous processes are still prevailing. A most promising approach to counteract such shortfalls is the exploitation of biofilms for producing industrially relevant compounds. Biofilm formation is a common feature of microbes. Under certain conditions, they attach to various kinds of surfaces and form a sort of sessile community at aqueous solid interfaces [1]. Advantages of biofilm growing organisms as compared to their planktonic counterparts are their physical robustness, the ability to self-immobilize, and their long-term stability. In the recent years, we developed a number of different biofilm reactors and characterized the biofilm catalyst under reaction conditions. In this presentation, we will introduce three different biofilm reactor concepts and point out advantages and disadvantages. The basic configuration of a membrane attached biofilm reactor (MABR) turned out to be severely oxygen limited and difficult to scale up[2]. This shortcoming was circumvented by introducing a dual purpose ceramic membrane into the reactor system, which simultaneously served as growth surface for the biocatalyst and as aeration device[3]. This system is currently under evaluation for scale up.
In a novel approach, we combined a capillary three phase (aqueous-organic-gas) segmented-flow reactor with catalytic biofilms [4]. Based on the internal shear forces within such micro-capillary reactor systems, this set-up takes advantage of high mass transfer rates. In addition, these shear forces prevent the system from clogging and control the biofilm thickness. We will present data regarding the conversions of octane and styrene to octanol and (S)-styrene oxide, respectively, in these different set-ups and discuss the pros and cons of these approaches for biofilm based catalysis.
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OTV022

Growth-decoupled, anaerobic succinate production from glycerol with pyruvate-kinase deficient *E. coli* mutants

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We constructed *E. coli* strains for succinate production from glycerol by a rational combination of gene deletions and a concomitant evolutionary design. Based on elementary mode calculations the formation of 1 mol succinate from 1 mol glycerol with simultaneous fixation of 1 mol of CO_2 represents the theoretical maximum yield. This can be realized if succinate is exclusively formed by PEP carboxylation followed by the reductive branch of the tricarboxylic acid cycle. Therefore the genes *pykF* and *pykA*, both encoding pyruvate kinases, were deleted. Otherwise, the pyruvate kinases would catalyze the direct conversion of PEP into pyruvate. The resulting strains could, however, barely grow on glycerol, presumably caused by a certain pyruvate shortage. Only after a selection procedure for faster growth, the evolved mutants revealed growth rates in the range of 0.3 h^{-1}. In these strains, pyruvate was most likely formed through a novel pathway. It is proposed to be based on a complete 'rerouting' of metabolism which includes the following steps: PEP carboxylation to oxaloacetate, conversion of oxaloacetate to malate, and decarboxylation of malate to pyruvate, further termed *POMP*. Evidence for the *POMP*-pathway comes from the deleterious effects on growth after further deletion of genes coding for malic enzymes. The strain ss279 (Δ*pykA* Δ*pykF* Δ*gldA* Δ*ldhA* Δ*poxB* Δ*pflB* Δ*tdcE*) was finally used in a 'zero-growth cultivation' setup (direct biotransformation from glycerol to succinate) at a cell concentration of 0.4 g/L (DCW). To implement this, the generation of cell mass was aerobically assured, prior to the ultimate production phase, since *E. coli* is not able to grow anaerobically on glycerol, as long as a necessary external electron acceptor is absent. Consequently, succinate was produced from glycerol and carbon dioxide (or bicarbonate) in an adjacent anaerobic production phase at non-growing conditions. At initial lab-scale, we observed continuous succinate production over a period of 6 days. Herein, 58 mM glycerol was consumed and 48 mM succinate was produced, which corresponded to an average molar yield of 82 %. This indicated a net fixation of CO_2 in the production phase, which was further confirmed by stable isotope labelling assays, proving the incorporation of ^{13}C-labeled bicarbonate into the produced succinate.

OTV023

Gradual insight into *Corynebacterium glutamicum*`s central metabolism for the increase of L-lysine production

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Corynebacterium glutamicum is used for the large production of amino acids like L-glutamate, L-valine or L-lysine, the latter made in a scale of 8x10^5 annual metric tons. We applied a stoichiometric model and identified citrate synthase (CS) as most promising target to increase L-lysine production. We therefore replaced the two promoters which we identified in front of the CS gene gltA of a lysine producer by nine promoters of decreasing strength. The resulting set of strains was subsequently analysed with respect to CS activity, growth, and L-lysine yield. The decrease of CS-activity below 30% led to an increase in L-lysine yield accompanied by a decrease in growth rate. A reduced CS-activity of 6% produced an increase in L-lysine yield from 0.17 g/g to 0.32 g/g. As a further step the global consequences at the transcriptome, metabolome, and fluxome level were monitored within the strain series. Reduced CS activity results in altered expression of genes controlled by RamA and RamB, and increased cytosolic concentrations of aspartate and aspartate-derived amino acids. The fluxome study revealed that reduced CS-activity surprisingly has only a marginal influence on CS flux itself, but increases the internal concentration of its substrates oxaloacetate and acetyl-CoA, thus showing that the observed systemwide macroscopic effects are due to locally bordered differences.
This systemic approach opens an exciting new view on the system *C. glutamicum* as an excellent and robust producer of bulk compounds and raises new challenges for stoichiometric models applied to the living cell.

OTV024

Induction of systemic resistance in soybean by the antagonistic epiphyte *Pseudomonas syringae* 22d/93

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The use of naturally occurring antagonists to suppress plant diseases offers an alternative to classical methods of plant protection. The epiphyte *Pseudomonas syringae* 22d/93, isolated from a healthy soybean leaf, shows great potential for controlling *P. syringae* pv. *glycinea*, the causal agent of bacterial blight of soybean. Its activity against *P. syringae* pv. *glycinea* is highly reproducible even in field trials, and the antagonistic mechanisms involved are of our special interest. It has been proposed that several attributes contribute to biocontrol, including antibiosis, competition for nutrients, niche exclusion, interference with cell signalling systems, and induction of systemic plant resistance.
We used Affymetrix soybean genome arrays to examine transcriptional changes that occur in soybean leaves inoculated with the antagonist *P. syringae* 22d/93. If the antagonist is able to trigger plant defence mechanisms prior to infection by the pathogen, disease can be reduced. The resulting elevated state of resistance in plant parts distant from the site of primary triggering is variably referred to as systemic acquired resistance (SAR) or induced systemic resistance (ISR). SAR is characterized by an early increase in salicylic acid, which appears to be an essential signalling molecule in the SAR pathway and by the accumulation of pathogenesis-related (PR)-proteins. ISR is induced by non-pathogenic organisms, e.g. plant growth-promoting rhizobacteria and depends on the plant signalling molecule jasmonic acid. Infiltration of soybean leaves with *P. syringae* 22d/93 led to the up- or down-regulation of more than 2.800 genes, respectively, more than twofold as compared with control plants. Noteworthy, several genes encoding PR-proteins and genes involved in phytoalexin production were up-regulated, indicating that *P. syringae* 22d/93 can induce SAR in soybean.

OTV025

Anaerobic denitrifying methane oxidation in a deep oligotrophic freshwater lake

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Denitrifying methane oxidation has first been described in 2006 for an enrichment culture originating from an eutrophic freshwater habitat. Bacteria of subgroup a of the NC10 phylum are proposed to carry out this novel process. However, nothing but scarce sequence information and one report on denitrifying methane oxidation in a wastewater treatment plant is available on the distribution of this process and the respective bacteria in the environment.
To assess the importance of denitrifying methane oxidation in natural habitats, we investigated the occurrence of this process and the distribution of the respective NC10 bacteria in sediments of Lake Constance. Radiotracer experiments were performed to track the process in littoral and profundal sediments and the diversity of NC10 bacteria was analyzed using molecular methods.
Denitrifying methane oxidation was reliably detected only in profundal sediments, but rates were about 20 times lower than aerobic methane oxidation rates in these experiments. After those indications for a spatial distribution pattern of this process, the community composition and distribution of NC10 bacteria were investigated in greater detail and at higher spatial resolution. NC10 bacteria of group a, the denitrifying methanotrophs, were not detectable in Lake Constance sediments at shallow water sites (<5 m), but marker genes were easily amplified from profundal samples (>80 m), indicating that littoral sediments do not provide a suitable habitat for these bacteria.
More studies on different habitats are needed to estimate the role of denitrifying methane oxidation in the global carbon and nitrogen cycle, but here we present first evidence for the occurrence this process in deep oligotrophic lakes and revealed a clear spatial distribution pattern of the responsible microorganisms.

OTV026

Life inside the nucleus - an unusual symbiont of amoebae related to rickettsiae

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Free-living amoebae are abundant in various habitats worldwide and are among the most important predators of microorganisms. Some bacteria, however, are able to evade phagocytosis by amoebae and may establish a stable and symbiotic relationship with these protozoa. Protozoa have thus been suggested to play an important role for the evolution of intracellular bacteria including human pathogens. From a nitrifying bioreactor we recently isolated a free-living amoeba strain identified by 18S rRNA gene sequencing as *Hartmannella* sp. These amoebae were stably infected with bacteria that unexpectedly were located inside the host nucleus, as demonstrated by fluorescence in situ hybridization and electron microscopy. All known amoeba symbionts live in the host cytoplasm; an endonuclear symbiont has not been described, and such a life style is also rare in other eukaryotic hosts. Phylogenetic analysis of these bacteria, named FS-5, revealed an only low degree of 16S rRNA sequence similarity (89 %) to their closest relative, the paramecium symbiont *Caedibacter caryophilus*. Endonuclear symbiosis requires a complex infection process. We showed that FS-5 is not limited to *Hartmannella* hosts but can also infect *Acanthamoeba castellanii*. We studied the infection process and the developmental cycle in both hosts by fluorescence in situ hybridisation combined with DNA staining and assessed host fitness by propidium iodide staining and determination of amoeba cell numbers. Hardly any bacteria could be detected in the cytoplasm at early time points, suggesting that the bacteria with a high degree of specificity traffic to the host nucleus. High infection levels were reached after 120 h, at which time point the nucleus is pronouncedly enlarged and completely filled with bacteria. Interestingly, there is no obvious deleterious effect on the amoeba hosts during the first 120 h post infection, but host cell lysis was observed at later time points. Presently, further experiments regarding the host range of FS-5 and its distribution in the environment, as well as genome sequence analysis is underway. This will help elucidate the molecular mechanisms underlying the endonuclear lifestyle of this unique symbiont.

OTV027

Functional community analysis of a microbial mat involved in the oxydation of iron by metatranscriptomics

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Through the capacity to use Fe(II) as an electron source and the conservation of energy by this process neutrophilic iron oxidizing microorganisms (FOMs) play an important role in iron redox cycling. While the role of the biotic and abiotic process was seen controversially for a long time, new data confirming the importance of microbial implication in this process are accumulating. The typical characteristic of described FOMs living at circumneutral pH is their lithotrophic metabolism using iron as their sole energy source. To compete with abiotic iron oxidation this oxygen-dependent reaction must take place at the oxic-anoxic interface, conditions often only found in difficult accessible microenvironments masked by complex ecosystems as soils and sediments. The recent detection of neutrophilic FOMs in a large variety of environments, as sediments, iron seeps, wetland soils and rhizosphere, bear testimony to their wide distribution and their importance in global iron redox cycling. While most oxic-anoxic interfaces are difficult to access and to analyze, FOMs are flourishing at the particular redox boundary characterized by steady fluxes of Fe(II) originating from an anoxic source and the oxygen is supplied from an oxygenated water body. These conditions are given and most obvious visible for example in iron-rich microbial mats, spanning often several tenths of meters with various depths. This model implies several ecological questions in particular about the multi- partnership mutualism and syntrophy, the coupling of the diversity with the function of the microbial actors and the long-term maintenance of the mat. The major objective of our project is to understand the role of the interactions among microorganisms in the biogeochemical functioning of an ecosystem involved in iron redox cycling. The activities of the microbial community were analyzed by comparative metatranscriptomics and correlated to biogeochemical factors. The analysis of the microbial diversity revealed the activities of a relative limited number of species. The most abundant active microorganisms were affiliated only to three different groups: the Alveolata (eucaryote), the methanotrophs (gammaproteobacteria) and the betaproteobacteria potentially involved in iron oxidation. While the presence of iron oxidizer were expected, the activity of methanotrophs was surprising. The statistical analysis showed a vertical spatial structuring of the microbial community in dependence of the depth with higher activities of methanotrophs near the sediment. These results indicate that the stability and the structuring of the mat is based on functional interactions among methanotrophs and iron oxidizing bacteria. Our study shows that in-depth metatranscriptomic approaches allow the linkage of the microbial diversity to function as well as the linkage of the microbial activity to environmental factors.

OTV028

Testing the limits of 454 pyrotag sequencing: reproducibility and quantitative assessment

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Characterization of microbial community structure via 16S rRNA gene profiling has been greatly advanced in recent years by the application of amplicon pyrosequencing. The possibility of barcode "tagging" sequencing templates gives the opportunity to massively screen multiple samples from environmental or clinical sources for community details.
However, an on-going debate questions the reproducibility and semi-quantitative rigour of pyrotag sequencing, and, as in the early days of genetic community fingerprinting, pros and cons are continuously provided.
In this study we investigate the reproducibility of bacterial 454 pyrotag sequencing over biological and technical replicates of natural microbiota. Moreover, via quantitatively defined template spiking to the natural community, we explore the potential for recovering specific template ratios within complex microbial communities. For this reason, we pyrotag sequenced three biological replicates of three samples, each belonging from yearly sampling campaigns of sediment from a tar oil contaminated aquifer in Düsseldorf, Germany. Furthermore, we subjected one DNA extract to replicate technical analyses as well as to increasing ratios (0, 0.2, 2 and 20%) of 16S rRNA genes from a pure culture (Vibrio fisheri) originally not present in the sample.
Unexpectedly, taxa abundances were highly reproducible in our hands, with max standard deviation of 4% abundance across biological and 2% for technical replicates. Furthermore, our workflow was also capable of recovering V. fisheri amendmend ratios in reliable amounts (0, 0.29, 3.9 and 23.8%). These results highlight that pyrotag sequencing, if done and evaluated with due caution, has the potential to robustly recapture taxa template abundances within environmental microbial communities.

OTV029

Microbial Communities of Marine Methane Seeps: Sketching the Big Picture

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Global ocean sampling efforts like the GOS expedition[1] and the International Census of Marine Microbes (ICoMM)[2] have revealed distinct microbial communities in surface and deep waters, coastal and open ocean ecosystems as well as in pelagic and benthic realms[3]. This presentation aims at sketching the big picture of archaeal and bacterial communities inhabiting cold seeps. We have analyzed 26 methane seep ecosystems of different temperature regimes across all major oceans from the Arctic to Antarctica. To identify the full range of resident methanotrophic key players as well as microbial taxa within the rare biosphere, 454-pyrosequencing of the variable region V6 within the 16S rRNA gene was applied. In addition to a description of biogeography, community composition, ß-diversity and covariation of certain taxa, environmental data were included in order to explain some of the emerging patterns. First results indicate that the seep communities are far more diverse and distinct than previously assumed. 80% of the archaeal OTUs (operational taxonomic units at a 97% nucleotide similarity cut-off) belonged to a variable community occurring at some seeps, but not at others. Interestingly, this variable community included all anaerobic methanotrophic (ANME) key players with the ANME-2a/2b clade being most widespread. Around 18% of archaeal OTUs were unique (occurring only at one seep) and only 2% of archaeal OTUs were residents (occurring at all seeps). These residents were identified as organisms of the Miscellaneous Crenarchaeotal Group and Marine Benthic Group B. The bacterial rare biosphere was even more prominent with 30% of all bacterial OTUs being unique and only about 1% of resident OTUs. The remaining 69% of all OTUs, the bacterial variable community, were dominated by *Delta-* and *Gammaproteobacteria*, while most of the bacterial residents are yet unknown, since they could merely be classified to the order level and lacked cultivated representatives. This presentation will discuss ways to define core microbial communities of marine methane seeps in distinct ocean realms and main factors driving their diversity.

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OTV030

Prokaryotic diversity in Pacific Ocean manganese nodules

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DNA extraction from different parts (hydrogenetic, diagenetic, core, edge) of a Pacific Ocean manganese nodule and a manganese crust collected during the cruise SO205 in 2010 with the German research vessel Sonne were analyzed with qPCR and via clone libraries for 16S rRNA genes of Archaea and Bacteria. Results indicate highest cell numbers in the diagenetic and circular edge nodule parts with about 1.7×10^8 - 3×10^8 cells/g and similar values (1×10^8 cells/g) were obtained for the top 2 cm of the nodule surrounding sediment. Cell numbers inside the nodule and in the hydrogenetic part were lower by one order of magnitude (about 10^7 cells/g). Bacterial cell numbers were always higher than numbers of Archaea. The diversity (Yue & Clayton Q_{YC} similarity coefficient) of bacterial and archaeal communities associated with the nodules was different from the community diversity in the sediment and on the manganese crust. Bacterial species highly similar to Shewanella benthica were found in all clone libraries from the nodule but not in the surrounding sediment or in the manganese crust. Nearly all obtained archaeal 16S rRNA gene sequences belonged to the Marine Group I Crenarchaeota.

OTV031

Inhibition of heterotrophic bacteria by solar radiation in a humic lake

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Light excitation of colored dissolved organic matter (cDOM) lead to photochemical reactions that produce low molecular weight (LMW) growth substrates that stimulate bacterial activity and inhibitory reactive oxygen species (ROS). In order to investigate the impact of ROS generation on bacterial activity we monitored diurnal cycles of ROS formation and bacterial activity in the humic south-west basin of Lake Grosse Fuchskuhle. High solar radiation caused strong inhibition of bacterial ^{14}C-leucine and ^{14}C-acetate uptake in surface waters and increased the fraction of membrane-damaged cells assessed by life/dead staining. The inhibition was paralleled by the formation of ROS, which very likely are the agents causing bacterial inhibition. In order to verify our data, cultures representing predominant bacterial phylotypes of the SW basin were incubated in the surface water layer by using dialysis bags. Acetate and leucine uptake and the fraction of membrane-damaged cells were monitored in those cultures. *Novosphingobium acidiphilum* (*Alphaproteobacteria*) represents a persistent species of the SW basin and was not hampered in activity by solar radiation. In contrast, the activity of *Polynucleobacter necessarius* a predominant *Betaproteobacteria* representative was strongly inhibited by high solar radiation as indicated by a low uptake of acetate and leucine compared to early morning samples. Cultures of both strains showed a very high fraction of life cells that did not decrease during daytime hours. Hence, we conclude that *N. acidiphilum* and *P. necessarius* have efficient mechanisms to cope with inhibitory products of photochemical reactions with respect to maintenance of cell integrity. Interestingly, solar radiation mediated formation of inhibitory substances leads to very low activity of *P. necessarius*, but not of *N. acidiphilum*. Hence, photochemical reactions that generate inhibitory ROS affect predominant bacteria of a humic lake in a species-specific manner.

OTV032

Phylogenetic characterization and comparison of microbial communities in mesophilic and thermophilic anaerobic digesters

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Mesophilic (30-40°C) and thermophilic (45-60°C) anaerobic digestion of substrate are the two main processes for biogas production. Mesophilic digestion is the most commonly used process with higher operating robustness, while thermophilic digestion provides higher biogas production with improved hygiene by reducing the pathogens. The largely unknown compositions of microbial communities, especially the hydrolytic bacterial communities involved in these processes are the key to understand the complex process. Thus cost-effective process could be chosen under different circumstances.
Lab scale mesophilic (~38.8°C) and thermophilic (~55°C) digesters operated with energy plants with semi-continuous stirring and daily feeding were used in this study. Bar-coded amplicon pyrosequencing of

16S rRNA genes was applied to acquire an overview about composition of microbial communities in both types of digesters. Hydrolytic microbial communities were characterized applying a functional oligonucleotide microarray targeting 756 different cellulase genes.
The pyrosequencing results showed that members of the phylum Firmicutes dominated in both digesters ranging from 73.8-84.5%. Representatives of the phylum Actinobacteria were the second abundant group found in both digesters, with a lower proportion detected in the thermophilic one. Significant differences in microbial community structures of the two digesters were found at finer taxonomical levels. The functional oligonucleotide microarray focuses on the detection of four different cellulase gene families and results revealed similar functional distribution patterns between the two types of digesters although a higher diversity within the cellulolytic microbial community was found in mesophilic digesters.
In conclusion, this study could show that the operating conditions did affect the diversity of microbial community, while the effect on the hydrolytic bacterial communities involved seems to be less pronounced.

OTP001

Molecular cloning of enantioselective ester hydrolase from *Bacillus pumilus*

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A gene from Bacillus pumilus expressed under its native promoter was cloned in Escherichia coli. Recombinant B. pumilus esterase (BPE) affects the kinetic resolution of racemic mixtures such as unsubstituted and substituted 1-(phenyl)ethanols (E ~ 33–103), ethyl 3-hydroxy-3-phenylpropanoate (E ~ 45–71), trans-4-fluorophenyl-3-hydroxymethyl-N-methylpiperidine (E ~ 10–13) and ethyl 2- hydroxy-4-phenylbutyrate (E ~7). The enzyme is composed of a 34-amino acid signal peptide and a 181-amino acid mature protein corresponding to a molecular weight of ~19.2 kD and pI ~ 9.4. 3-D the structural model of the enzyme built by homology modelling using the atomic coordinates from the crystal structure of B. subtilis lipase (LipA) showed a compact minimal a/b hydrolase fold.
Biography: Prof. V. Verma, a former scientist of Indian Institute of Integrative Medicine (formerly known as Reg. Res. Lab), Jammu , a CSIR research laboratory and presently Professor of Biotechnology, Shri Mata Vaishno Devi University, Katra (J&K) is acclaimed for his work in microbial biotechnology. His work in this field mainly related to the cloning & heterlogous over-expresion of microbial genes encoding enantio-specific enzymes known for resolving the racemic drug intermediates. Besides, he did pioneering work in the development of fermentation based technologies for the mass production of selected microbial isolates as biocontrol agents as part of integrated nutrient & disease management of agriculturally important plants. His research areas include microbial gene cloning & their heterologous expression, fermentation technology for organic agriculture and DNA finger printing of the important microbial isolates for IPR & registration purposes.Prof. Verma is recipient of a number of national/international awards in Biological Sciences and Biotechnology. He is Fellow of a number of Academies of Sciences in India. Recently he received INDUSTRIAL MEDAL AWARD from the Biotech Research Society of India for his outstanding contributions in Biotechnology.

OTP002

Isolation and characterization of novel potent Cr (VI) reducing alkaliphilic bacterim from hypersaline soda lakes

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A strain KSUCr3 with extremely high Cr(VI)-reducing ability under alkaline conditions was isolated from hypersaline soda lakes and identified as Amphibacillussp. on the basis of 16S rRNA gene sequence analysis. The results showed that Amphibacillussp. strain KSUCr3 was tolerant to very high Cr(VI) concentration (75 mM) in addition to high tolerance to other heavy metals including Ni^{2+} (100 mM), Mo^{2+} (75 mM), Co^{2+} (5 mM), Mn^{2+} (100 mM), Zn^{2+} (2 mM), Cu^{2+} (2mM) and Pb (75mM). Strain KSUCr3 was shown to be of a high efficiency in detoxifying chromate, as it could rapidly reduce 5 mM of Cr(VI) to a non detectable level over 24 h. In addition, strain KSUCr3 could reduce Cr(VI) efficiently over a wide range of initial Cr(VI) concentrations (1 -10 mM) in alkaline medium under aerobic conditions without significant effect on the bacterial growth. Addition of glucose, NaCl and Na_2CO_3 to the culture medium caused a dramatic increase in Cr(VI)-reduction by Amphibacillus sp. strain KSUCr3. The maximum chromate removal was exhibited in alkaline medium containing 1.5% Na_2CO_3, 0.8% glucose, and 1.2% NaCl, at incubation temperature of 40 ºC and shaking of 100 rpm. Under optimum Cr (VI) reduction conditions, Cr(VI) reduction rate reached 237 μMh^{1} which is one of the highest Cr(VI) reduction rate, under alkaline conditions and high salt concentration, compared to other microorganisms that has been reported so far. Furthermore, the presence of other metals, such as Ni^{2+}, Co^{2+}, Cu^{2+} and Mn^{2+} slightly stimulated Cr(VI)-reduction ability by the strain KSUCr3.The isolate,Amphibacillus sp. strain KSUCr3, exhibited an ability to repeatedly reduce hexavalent chromium without any amendment of nutrients, suggesting its potential application in continuous bioremediation of Cr(VI). The results also revealed the possible isolation of potent heavy metals resistant bacteria from extreme environment such as hypersaline soda lakes.

OTP003

α-amylase production by *Bacillus* species isolated from sweat food waste

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Industrial applications of enzymes have been receiving attention throughout the world. Amylases are of great importance in biochemical processes, and wide range of application of amylases have used in various sectors like confectionary, baking, paper, textile, detergent, beverages, baby foods, medicinal and pharmaceutical manufacturing industries which drew both researchers and industry excessive attention.
It is became a routine work to isolate and produce amylase from different fungal sources, however, the current work aimed to produce amylase enzyme from bacterial source (Bacillusspecies). In order to do that, three different formulas has been chosen, the first one is only glucose (starch free), the second one is mixture of glucose and starch, and the last one is only starch (with six isolation), at pH 7 (±0.2) and (37°C). The production activity has been measured by spectrophotometer in each formula at until 8hours time intervals.
The results obtained from formula I showed no significant change in the level of glucose and this is because of the gene coding of amylase activity was turned off (enzyme repression) as a result of glucose availability. Formula II showed modest decrease in glucose concentration because the bacteria used the free glucose available rather than breaking up the starch to get glucose. However, in formula III there was an increase in the level of glucose concentration especially after one hour of incubation as a result of amylase enzyme activity.
Finally, formula III proved the production of α-amylase from *Bacillus* species isolated and identified from sweat food waste.

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OTP004

Identification of acetate incorporating *Arcobacter* spp. as potential manganese reducers in pelagic redoxclines of the central Baltic Sea via 16S rRNA based ^{13}C stable isotope probing

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Pelagic redoxclines in the central Baltic Sea are recognized as environments with elevated microbial activities comprising both, heterotrophic and autotrophic prokaryotes, involved in important biogeochemical cycles. Aim of our study was to reveal first insights into the identity and function of heterotrophic bacteria in this habitat which is well-studied with respect to autotrophic activities. Therefore, pelagic redoxclines of the Gotland basin were sampled in 2005 and 2009, respectively, and subjected to stimulation experiments with different organic substrates and electron acceptors, followed by the identification of stimulated bacteria using 16S rRNA gene single strand conformation polymorphism (SSCP) analyses. In addition, RNA stable isotope probing (RNA-SIP) followed by subsequent 16S rRNA based quantitative RT-PCR and fingerprinting served to identify acetate incorporating organisms. In 2005, in water from the sulfidic zone, 17.3 μMol Mn^{4+} were reduced after 48 h and bacteria affiliated with the epsilonproteobacterial *Arcobacter* sp. dominated the incubation. In 2009, bulk incorporation of ^{3}H labelled acetate was highest in the oxic-anoxic interface layer and still high in the sulfidic zone. After 72 hours, bacteria affiliated with *Arcobacter* sp. incorporated the ^{13}C-labeled acetate in the oxic-anoxic interface layer and the sulfidic zone while the gammaproteobacterial genera *Neptunomonas* sp. and *Colwellia* sp. incorporated acetate in the oxic-anoxic interface layer only. Together, in both experiments two phylogenetically distinct clusters within the genus *Arcobacter* sp. were identified related to previously recovered *Arcobacter* sp. from manganese-oxide rich shelf

sediments in the Black Sea by Thamdrup *et al*. 2000. Thus, we identified acetate utilizing *Arcobacter* spp. as potential heterotrophic manganese reducers in pelagic Baltic Sea redoxclines.

OTP005

Artificial fusion of a two-component styrene monooxygenase

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Styrene monooxygenases (SMOs) are external flavoprotein monooxygenases performing enantioselective oxygenations of styrene and structurally related compounds (Montersino et al., 2011). In most cases a single NADH-dependent oxidoreductase (StyB) provides reduced FAD for a single styrene-epoxidizing monooxygenase (StyA). Recently, a remarkable reductase type StyA2B was found and biochemically characterized, in which the oxygenase subunit is naturally fused to the FAD reductase (Tischler et al., 2009). This wild-type single-component SMO was shown to be self-sufficient but of rather low epoxidation activity. Cooperation with another single styrene oxygenase subunit StyA1 is likely to be necessary to generate high specific epoxidation activity in host strain *R. opacus* 1CP (Tischler et al., 2010). Despite a current lack of knowledge on the biochemical reasons for the evolution of StyA2B, one-component SMOs may have several advantages over multicomponent systems as shown for other monooxygenase classes.
Herein, we describe the construction of self-sufficient chimeric styrene monooxygenases by a molecular genetic approach as well as the preliminary characterization of the recombinant proteins. The artificial fusion of the genes *styA* and *styB* from *Pseudomonas fluorescen*s ST by elimination of the stop codon of *styA* and by introducing various linker sizes (Lx) provided four *styALxB*-gene variants. The artificially fused one-component SMOs were successfully expressed in *E. coli* BL21. Activity was demonstrated from these clones by converting indole into indigo and later on determined by means of homogeneous protein preparations.
The artificial fusion of two-component SMOs was for the first time succesfully demonstrated and should provide access to valuable biocatalysts in the field of fine chemical syntheses.

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OTP006

Molecular basis of symbiosis investigated in *Chlorochromatium aggregatum*

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The phototrophic consortium "*Chlorochromatium aggregatum*" is a mulitcellular association between the green sulfur bacterial epibionts *Chlorobium chlorochromatii* and a central motile chemotrophic Betaproteobacterium. The flagellated central rod moves the entire consortium towards the light enabling the epibiont to conduct anoxygenic photosynthesis. The cells are connected through specific cell-cell adhesion structures and division results in two intact daughter consortia. These observations suggest the exchange of multiple signals between the epibiont and the central bacterium making this culturable association a suitable system for understanding the molecular basis of symbiosis between nonrelated bacteria. The comparison of the *Chl. chlorochromatii* genome with eleven available genomes of free-living relatives revealed unique open reading frames. The major fraction of the ORFs code for hypothetical proteins, but putative large exoproteins and a protein with a RTX toxin-type ß-roll were identified. In particular Cag1919 which bears several RTX repeats which are typically found in Gram-negative pathogenic bacteria is of interest. These putative symbiosis genes (Cag1919, Cag1920, Cag0614 and Cag0616) are constitutively transcribed and have been analysed further. The whole gene of Cag1919 was cloned into a vector of the pQE series and expressed heterologously in the *E.coli* strain *XL1Blue*. The protein can be used in Ca^{2+}- binding experiments due to its predicted Ca^{2+}-binding region. Cag0614 and Cag0616 represent the largest open reading frames in the prokaryotic world known to date with length of 110418 and 61938 bp, respectively. Due to their large size only fragments can be cloned and expressed. Interestingly, expression of Cag1919 and 1920 were deleterious to *E. coli* strains causing the formation of extremely long, filamented or branched cells. To facilitate the localization of the proteins in *Chl. chlorochromatii*, in the free-living and symbiotic state, the resulting recombinant proteins are used to produce antibodies for immunogold labelling and tyramide signal amplification. With these results proteins relevant in bacterial symbiosis can be localized and the question how a motif known from pathogenic bacteria operates in symbiosis approached.

OTP007

Effect of phosphate on a community of iron oxidizing bacteria

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At the open pit Nochten (Lusatia, East Germany) mine waters are biotechnologically treated in a pilot plant by microbial iron oxidation with the subsequent precipitation of schwertmannite. These waters are characterized by a low pH value, high concentrations of iron and only traces of phosphate. The low phosphate availability appears to be a factor that influences the microbial activity. To increase the capacity of the pilot plant, the effect of phosphate on the oxidation rate and the microbial community was investigated.
The microbial community of the treatment plant was cultivated with and without phosphate in a pilot plant-adapted laboratory set up. Besides the determination of chemical parameters like pH, iron and phosphate concentration the microbial community was quantitatively characterized by cell counting and qualitatively by T-RFLP analysis. The results show that the iron oxidation rate, the bacterial community and cell numbers differed significantly based on the phosphate availability. The cell number was doubled in the system with phosphate. The microbial community in the reactor without phosphate consisted of various iron oxidizing (60%) and non-iron oxidizing bacteria (40%), whereas exclusively the iron oxidizing bacteria '*Ferrovum myxofaciens*' was present in the reactor with added phosphate.
Together with the higher cell number and higher percentage of iron oxidizer in presence of phosphate also the oxidation rate increased compared to the system without phosphate.

OTP008

L-Sorbitol-Dehydrogenase from *Bradyrhizobium japonicum* USDA 110 can be applied in D-Sorbose Production using Electrochemical Cofactor Regeneration

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In the FP7 EU project ERUDESP an enzyme reactor with electrochemical cofactor regeneration was developed for the production of enantiopure building blocks used in pharmaceutical synthesis. Applying the suitable enzymes, the reactor can also be used for the production of rare sugars like D-sorbose, which is an interesting synthon for pharmaceutical applications and can be used as a low calorie sweetener. An annotated ribitol-dehydrogenase gene of *Bradyrhizobium japonicum* USDA 110 was identified in a BLAST search with the N-terminal amino acid sequence of an earlier described L-sorbitol-dehydrogenase of *Stenotrophomonas maltophilia* [1], which oxidizes L-sorbitol to D-sorbose. The gene was amplified, tagged with histidines and heterologously expressed in *E. coli* BL21Gold(D3). The biochemical examination of this protein exhibited comparable L-sorbitol-dehydrogenase activity to the S. maltophiliaenzyme. The L-sorbitol-dehydrogenase from *B. japonicum* was encapsulated together with diaphorase in sol-gel layers on gold electrodes as has been described earlier for a D-sorbitol-dehydrogenase from *Rhodobacter sphaeroides* [2]. With NAD^+ and the mediator ferrocenedimethanol in solution the oxidation of D-sorbitol could be demonstrated by cyclic voltammetry. The results let the enzyme appear as a promising candidate for the production of the rare sugar D-sorbose in enzyme reactors with electrochemical cofactor regeneration.

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OTP009

Biofouling of ultrafiltration membranes for drinking water treatment characterized by Confocal Laser Scanning Microscopy

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Biofouling is known as a major reason for flux decline in the performance of membrane based water and wastewater treatment plants. The relevance of biofilm extracellular polymeric substances (EPS) in terms of fouling on membranes has been indicated in several studies. Therefore, a profound knowledge of the composition of biofouling is important for the development of new countermeasures in enhancing membrane permeability.
The objective of this investigation was the characterization of microbial aggregates and EPS components in biofilms that contribute to biofouling of ultrafiltration membranes using confocal laser microscopy (CLMS).
Biofouling tests were conducted using an experimental setup, where a hollow-fiber ultrafiltration (UF) membrane module made of polyethylene was fed with natural water. Dead end filtration was carried out continuously by using a constant pressure of 20mbar and an initial

membrane permeability of 390$Lh^{-1}m^{-1}bar^{-1}$. One operation cycle consisted of 20min of filtration and a backwash of 20sec. Samples of fouled membranes were investigated after one, three and six cycles of filtration. The biofouling was analyzed by confocal laser scanning microscopy (CLSM) after simultaneous staining. The bacteria in the fouling were stained with DAPI specific to nucleic acids and different fluorescent labeled lectins specific to polysaccharides of the EPS.
Confocal laser microscopy showed that biofouling on the membrane was a composition of heterogeneous colonization of bacteria and extra cellular polymeric substances (EPS) containing, N-acetylglucosamine, N-acetylgalactoseamine and L fucose. The detection of the bacteria and the location of the polysaccharides could be related to the biofouling accumulation. Our investigations assume, that at first polysaccharides of the influent adsorbed to the membrane surface and serve as layer for the development of a conditioning film. Backwashing was able to remove cells from the membrane, but was unable to remove adsorbed substances of the conditioning film.

OTP010

Evaluation of analytical sensitivity and specificity of the biothreat assay for clinical *Bacillus anthracis* diagnostics by the PLEX-ID™ System

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Bacillus anthracis causes a clinical condition known as Anthrax disease and the bacterium is placed top on the list of biological agents potentially to be used in bioterrorism and biological warfare.*B. anthracis* belongs to the *B. cereus* group spp., which are genetically closely related. For instance, plasmids similar to *B. anthracis* pXO1 and pXO2 can also be found in *B. cereus*. These plasmids are of paramount importance for virulence of the bacilli. pXO1 codes for the toxins edema- and lethal-factor along with protective antigen needed for toxin delivery into host cells. pXO2 is required for capsule formation enabling evasion of host immune response. The PLEX-ID™ System is a technique based on PCR and Electrospray-Ionization Mass Spectrometry (ESI-MS) providing the exact base-composition of (partial) gene amplificates. As part of a so-called "biothreat assay" species specific primer sets were developed enabling the detection of 46 viral and bacterial biothreat pathogens.
Herein, closely related organisms can be differentiated in a single run on a multiplex-assay-base aiming at reliable and fast identification of unknown samples by (subspecies-) specific base pair signatures. *B. anthracis* detection, for example, is achieved via two *B. anthracis* specific chromosomal and one pXO1- and pXO2-plasmid specific targets. To evaluate this "biothreat assay" we tested its analytical specificity (cross-reactivity) and analytical sensitivity [limit of detection (LoD)]. For this, we analyzed a panel of *B. anthracis* (plasmid positive and negative) strains and *Bacillus* spp. isolates closely related to *B. anthracis*. Included in this study were also other organisms representing the resident flora of clinical matrices and various matrices relevant in clinical *B. anthracis* diagnostics. The LoD was as low as 5 genome copies per µl from culture and 5 to 10 genome copies from clinical matrices such as EDTA blood. Taken together, the PLEX-ID™ technique allows for the reliable identification of *B. anthracis* (plasmid positive and negative strains) and the discrimination from other *B. cereus*-group bacteria (incl. plasmid positive strains) within acceptable clinical sensitivity.

OTP011

Seeking novel Hydrogenases from a hydrothermal vent enrichment culture

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A culture enriched with diffuse fluids taken at the hydrothermal vent Sisters Peak (5° S on the Mid-Atlantic Ridge) grows autotrophically on artificial seawater supplemented with hydrogen. Analyses of amplified 16S rRNA genes revealed the presence of species commonly not known to utilize hydrogen as electron donor, namely the *Alphaproteobacterium Thalassospira sp.* and the *Gammaproteobacteria Thiomicrospira crunogena*, *Pseudomonas pachastrellae* and *Alteromonas macleodii*. Fluorescence *in situ* hybridization with specific probes designed to target each species individually demonstrated little community shifts in the culture within 4 weeks. The relative abundance of *T. crunogena* varied between 40-71% and of *Thalassospira sp.* between 25-40%, respectively. Relative abundances of *A. macleodii* and *P. pachastrellae* were between 2% and 10%. We also performed hydrogen consumption measurements with the enrichments, which clearly illustrated the active uptake of hydrogen. The uptake hydrogenase activity of membrane associated proteins from the mixed culture was 0.253 ± 0.079 µmol H_2*min^{-1}*mg^{-1}, contrasting the low uptake activity for the soluble proteins (0.023 ± 0.132 µmol H_2*min^{-1}*mg^{-1}). Conclusively, hydrogenases are being expressed and are active in this culture. Since we have not been able to assign the hydrogenases to one of the species in the enrichment culture we are currently pursuing 2 strategies: (i) Native PAGE and an in-gel hydrogenase activity assay in combination with sequencing of active protein bands and (ii) investigation of the isolated species with respect to growth with hydrogen, uptake hydrogenase activities and hydrogen consumption.

OTP012

Three-dimensional obstacles for bacterial surface motility

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Many bacterial species live at surfaces. For surface colonization they have developed mechanisms which allow them to move while remaining attached to surfaces. The most ubiquitous mode of surface motility is mediated by type IV pili. These polymeric cell appendages mediate motility through cycles of pilus polymerization, adhesion, and depolymerization. Natural adhesion surfaces, including mammalian host cells, are not flat. It is unknown, however, how the topography of a surface influences bacterial surface motility. Here, we show that the round Neisseria gonorrhoeae (gonococcus) was preferentially reflected from barriers with a depth of 1 µm but not by lower barriers. Gonococcal motility was confined to grooves whose dimensions were on the order of the size of the bacteria and the dynamics of movement was in agreement with a tug-of-war model. Likewise, the motility of the rod-like Myxococcus xanthus (myxococcus) was confined to grooves. In summary, the data demonstrate that surface-motile bacteria can sense the topography of the surface and that their movements are guided by microscopic elevations.
Meel, C., Kouzel, N., Oldewurtel, E.R., Maier, B. Three-dimensional obstacles for bacterial surface motility, Small, accepted.

OTP013

Recombinant production of genetically modified S-layer proteins in different expression systems

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Surface layer (S-layer) are proteins which cover the outermost of many prokaryotes and are probably the basic and oldest forms of bacterial envelope. These proteins are mostly composed of protein and glycoprotein monomers and have the ability to self-assemble into two-dimensional arrays on interfaces. Several characteristics like their work as molecular sieve, as virulence factor or the protection of the cell from toxic heavy metal ions make S-layer proteins interesting for their usage as ultrafiltration membranes, drug microcontainers, filter materials or patterning structures in nanotechnology.
Heterologous expression of S-layer proteins is not simple and depends on the used vector and the expression system. Equally the S-layer protein size, genetic specifics, and the existence of adapted signal peptides influence the expression. To enable an efficient and economical protein production protein secretion is the most favoured method.
In this work we describe the recombinant production of different S-layer variants and characterize the differences of the used protein expression systems.
We used four different S-layer genes of *Lysinibacillus sphaericus* JG-A12, *Bacillus* spec. JG-B12 and *Lactobacillus acidophilus* and expressed their proteins in *Escherichia coli*, *Pichia pastoris* and *Lactococcus lactis*. Some of these proteins were genetically modified to adapt the construct to the used S-layer expression system.
Our work identified *Lactococcus lactis* as the best expression system for the used S-layer genes.

OTP014

Biological applications for nano-mechanical detection ofmolecular recognition

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Advances in carbohydrate sequencing technologies have revealed the tremendous complexity of the glycome. Understanding the biological function of carbohydrates requires the identification and quantification of carbohydrate interactions with biomolecules. The increasing importance of carbohydrate-based sensors able to specifically detect sugar binding molecules or cells, has been shown for medical diagnostics and drug screening. Our biosensor with a self-assembled manno side based sensing

layer that specifically detects carbohydrate-protein binding interactions (mannoside - ConA), as well as real time interaction of carbohydrates with different E. coli strains in solution. Binding to the Cantilever surface causes mechanical surface stress, that is transduced into a mechanical force and cantilever bending. The degree and duration of cantilever deflection correlates with the interaction‘s strength. In this study we present carbohydrate-based cantilever biosensors as a robust, label-free, and scalable method to analyze carbohydrate-protein and carbohydrate-bacteria interactions. The cantilevers thereby exhibit specific and reproducible deflection with a high sensitivity range of over four orders of magnitude.

OTP015

Antibiotics Screening 2.0 - Tools for *in silico* Genome Mining for Natural Product Biosynthesis Pathways

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Microorganisms are a rich source for natural products of which many have potent antimicrobial or antitumor activity. While in the past, functional screening approaches directed directly to the substances or to putative targets were the main approaches for the identification and isolation of novel compounds, the easy availability of whole genome sequence data of putative producers nowadays offers great possibilities to assess the genetic potential of the strainsin silico.
For such analyses of genomic data novel, sophisticated tools are required which allow the prediction of putative biosynthetic products. Therefore, several tools were developed in our group:
The Open Source annotation platform **CLUSEAN**[1] is a versatile tool for the analysis of single biosynthetic gene clusters as well as whole genome sequences. CLUSEAN contains generic modules for automated BLAST or HMMer analyses as well as specialized tools for the domain assignment and specificity prediction of modular polyketide synthases (PKS) and non ribosomal peptide synthetases (NRPS).
Included into CLUSEAN is **NRPSpredictor**[2,3]. This tool allows the prediction of substrate specificities of adenylation domains of NRPS enzymes and thus the prediction of the peptide products. Here, we present the new version **NRPSpredictor**[2] which contains updated models for the amino acids and now allows prediction up to the amino acid level.
All of these tools are now also integrated into the antibiotics and secondary metabolites analysis shell **antiSMASH**[4]. This pipeline contains most tools that are currently available for the analysis of secondary metabolite gene clusters, including CLUSEAN and NRPSpredictor2. antiSMASH is either available as a standalone application or as a web based service.
URLs for downloading/using the software:
CLUSEAN: http://redmine.secondarymetabolites.org/projects/clusean
NRPSpredictor2: http://nrps.informatik.uni-tuebingen.de
antiSMASH: http://antismash.secondarymetabolites.org/

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OTP016

Relative protein quantification using ^{36}S- or ^{34}S- sulfate

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To uncover changes in the proteome and to draw conclusions from this it is crucial to quantify as accurate as possible. One of the favored methods is the metabolic introduction of stable isotopes. Currently in use are heavy labeled amino acids or substrates to directly compare the intensities of associated peptide pairs of two or more different conditions during a single measurement [1]. Even though these techniques have proven to be feasible, they have drawbacks as well. The addition of amino acids might influence the proteome or they get metabolized, resulting in an unpredictable spread of the label. The labeling of the whole proteome by ^{13}C or ^{15}N labeled substrates usually results in incorporation patterns which are hard to predict and therefore bioinformatically complicated [2].
Here we show the potential of utilizing heavy sulfur isotopes for relative protein quantification. Sulfur is an essential element for microorganisms and is part of methionine and cysteine, so it can be used as universal label for quantitative proteomic studies. The fact that sulfur containing amino acids are encountered infrequently is a mixed blessing. Although only a small fraction of measurable peptides will give quantitative information, the incorporation patterns are well predictable in comparison to carbon or nitrogen labeling strategies. So far the relative proteomic change of *P. putida* with benzoate as carbon source was elucidated using ^{36}S-labeled sulfate [3]. It could be shown that this technique leads to the relative quantification of many relevant proteins. Due to the high costs and low availability of ^{36}S-sulfur or -sulfate, we further investigated the usage of ^{34}S-labeled sulfate. As most tryptic peptides contain only one sulfur atom, the mass shift of 2 Da corresponding to the ^{34}S-label is not enough to fully separate the isotopic patterns with routine resolutions. We are showing that the in silico separation of the isotopic pattern for relative quantification is possible, taking the monoisotopic peak as reference to simulate the correct distributions. The proteomic change in *P. fluorescens* during naphthalene degradation will be presented from a label switch experiment using ^{34}S-sulfate to first confirm the suitability of ^{34}S as universal label and second to identify relevant physiological changes besides the known.

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OTP017

Development of a functional screening method for novel [NiFe]-hydrogenases from metagenomes

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The interconversion between molecular H_2 and protons and electrons is extremely interesting for biotechnological applications because H_2 is one of the most promising renewable fuels. This reaction is catalyzed by enzymes called hydrogenases ($H_2 <--> 2\ H^+ + 2\ e^-$). The direction of this reaction depends on the redox potential of the components able to interact with the enzyme. One biotechnological application for hydrogenases is in fuel cells, where energy becomes available through the oxidation of H_2. Alternatively, hydrogenases are applicable for the biological H_2 production in electrochemical cells. One of the most crucial challenges in these biotechnological applications is to resolve the problem associated with the oxygen sensitivity of hydrogenases.
In hydrothermal deep sea vent habitats, hot hydrothermal fluids enriched with reduced inorganic compounds e.g. H_2 emit from the ground. As the ascending hydrothermal fluids come in contact with cold, oxygenated ambient seawater, mixing processes constitute habitats with steep physico-chemical gradients, e.g. habitats with high concentrations of H_2 and oxygen. With respect to these abiotic conditions, the rich energy source provided by H_2 oxidation and the large numbers of diverse H_2-oxidizing microorganisms, hydrothermal vents facilitate ideal conditions for seeking oxygen tolerant hydrogenases.
To identify and study novel oxygen tolerant hydrogenases we used metagenomic material from these habitats and constructed broad-host range fosmid libraries. Since heterologous expression of functional hydrogenases in the standard host *Escherichia coli* is difficult because complex interactions of maturation- and assembly proteins are often needed, we are establishing function based screenings with alternative heterologous hosts. Therefore, two new deletion mutants are currently being constructed: These are the *γ-proteobacterium Shewanella oneidensis* MR-1 and the *ε-proteobacterium Wolinella succinogenes*. Both organisms possess a single [NiFe]-hydrogenase and are promising candidates for establishing this functional screening method. A [NiFe]-hydrogenase deletion mutant of *Shewanella oneidensis* MR-1 (Δ*hyaB*) was developed successfully and we here report our first results of the conducted functional screen.

OTP018

Fate of elemental sulfur in coastal sediments and hydrothermal vents

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Zero-valence sulfur (ZVS) species such as elemental sulfur (S^0) and polysulfides are central intermediates in sulfur cycling at redox clines in marine and freshwater sediments. We found significant amounts of ZVS at the sediment surface of tidal flats in the German Wadden Sea. Also, large S^0 precipitates are covering the surface at a hydrothermal system in the Manus Basin/Papua-New Guinea. It is generally unknown, how microorganisms in these environments metabolize dissolved and particulate ZVS under different oxygen regimes. To investigate the bacterial community utilizing ZVS, we sampled native S^0 from geochemically diverse systems in the Manus Basin. Moreover, we exposed S^0 slabs as colonization surfaces in both coastal sediments and at hydrothermal vents for a period of 2-6 weeks. To identify key S-cycling

bacteria in situ, we used 16S rRNA clone libraries, 454-tag sequencing and CARD-FISH. S^0 slabs incubated in oxic sediment were mainly colonized by epsilonproteobacteria that were related to Sulfurimonas and Sulfurovum. Sulfate formation supported that epsilonproteobacteria are important ZVS-oxidizing organisms not only in hydrothermal systems and in OMZs but also in temperate marine sediments. S^0 slabs from the anoxic sediment were colonized mainly by probably S-disproportionating Desulfocapsa. We will compare the diversity of S^0-utilizing organisms from coastal sediments and from hydrothermal systems to look for differences and commonalities. Our data will provide detailed insights into the bacterial community involved in biogeochemical cycling of zero valence sulfur species in different habitats.

OTP019

Synthetic microbial production pathways for benzoyl-CoA-derived metabolites

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Benzoyl-CoA is a key intermediate in several metabolic pathways. For instance it is a precursor for polyketide synthesis in plants and microorganisms or an intermediate in aromatic hydrocarbon degradation. Since benzoyl-CoA normally does not occur in microbial metabolism, any designed benzoyl-CoA-dependent microbial pathway requires an initiation module to synthesise this intermediate. We made use of a benzoate transporter and a benzoate-CoA ligase from the betaproteobacterium *Aromatoleum aromaticum*, which are involved in anaerobic benzoate degradation, to create recombinant benzoyl-CoA-producing bacterial strains.
Our first efforts to couple benzoyl-CoA to the synthesis of a product aimed at the polyketide biphenyl, which is synthesised from benzoyl-CoA and three malonyl-CoA by biphenyl synthase of the rowan berry *Sorbus aucuparia*[1]. The initial host strain of E.coli did not produce any detectable products, probably because of limited malonyl-CoA supply. Therefore, we shifted to the related *Shimwellia* (formerly *Escherichia*) *blattae*, since this species can be grown on malonate[2], which should release the metabolic bottleneck. First results indicate that a metabolite is indeed produced by recombinant cultures fed with benzoate and malonate, which will be further characterized.
Another more extended possible biosynthetic pathway starting from benzoyl-CoA is pursued for the production of (*R*)-benzylsuccinate, an aromatic compound of potential interest for production of biodegradable polymers. (*R*)-benzylsuccinate is an intermediate of anaerobic toluene degradation and is usually synthesised from toluene and fumarate via a glycyl radical enzyme[3]. We will try to establish a synthetic pathway for this compound from benzoyl-CoA and the mixed acid-fermentation product succinate by reversing the β-oxidation pathway involved in benzylsuccinate degradation. The first step of this reverse pathway is the condensation of benzoyl-CoA and succinyl-CoA to benzoylsuccinyl-CoA by a new type of thiolase. First results on establishing this reaction will be shown.

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OTP020

Influence of the initial dissolved H_2 concentration on the reductive dechlorination of 1,2,3-trichlorobenzene by *Dehalococcoides* sp. strain CBDB1

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The genus *Dehalococcoides* comprises strictly anaerobic bacteria that conserve energy exclusively by organohalide respiration. They only use halogenated organic compounds such as 1,2,3-trichlorobenzene (TCB) as electron acceptor and hydrogen as electron donor. Moreover, they only use acetate as a carbon source. The genome of *Dehalococcoides* sp. strain CBDB1 encodes 32 homologous cobalt-dependent reductive dehalogenases (Rdh), 5 types of multi-subunit hydrogenases (Hyd) and a putative formate dehydrogenase (Fdh) as one of the most abundant proteins in strain CBDB1 [1].
The influence of varying the dissolved H_2 concentration on the stoichiometric dechlorination of 1,2,3-TCB to 1,3-dichlorobenzene (DCB) was monitored in *Dehalococcoides* sp. strain CBDB1 using gas chromatography. Initial dissolved H_2 concentrations from 1 μM to 10 μM were applied to cultures containing 50 μM 1,2,3-TCB. The complete dechlorination of 50 μM 1,2,3-TCB was achieved within 48 hours using an initial H_2 concentration of 10 μM. We could demonstrate that the rate of reductive dechlorination of 1,2,3-TCB to 1,3-DCB increased with increasing initial dissolved H_2 concentrations. Moreover, the hydrogen uptake rate also increased with increasing dissolved H_2 concentrations. Hydrogen consumption was also observed at an elevated redox potential (between -350 and -110 mV), whereas under these conditions reductive dechlorination did not occur. This indicates that hydrogen oxidation and reductive dechlorination can be uncoupled. Using qRT-PCR it could be demonstrated that the genes encoding the catalytic subunits of the Fdh and the periplasmatic hydrogenase Hup were highly expressed. Expression of these genes was also influenced in response to different initial dissolved H_2 concentrations.

Acknowledgement: This work is supported by the DFG (research unit FOR1530)
[1] Kube *et al.* (2005) Genome sequence of the chlorinated compound-respiring bacterium *Dehalococcoides* species strain CBDB1. Nat Biotechnol 23, p.1269-1273

OTP021

Heterologues Regulation of Reductive Dehalogenase Gene Expression by a MarR-type Regulator

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The anaerobic bacterium *Dehalococcoides* sp. CBDB1 belongs to the phylum Chloroflexi and is unusual in that it is able to dechlorinate different chloroaromatic compounds such as 1,2,3-trichlorbenzene (TCB) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Dechlorination takes place in a process called organohalide respiration and is catalysed by reductive dehalogenases (Rdh) and driven by hydrogen as electron donor. The genome of strain CBDB1 contains 32 genes encoding putative Rdhs [1]. The Rdh enzymes consist of a catalytic subunit RdhA together with a putative membrane-anchor subunit RdhB and these are encoded by *rdhAB* operons. The *rdhAB* operons are closely associated with genes encoding either MarR-type or two-component system (TCS) transcriptional regulators. The role of these regulators in the transcriptional control of the respective *rdhAB* genes is unclear.
Because *Dehalococcoides* bacteria are not genetically tractable and are difficult to work with, a heterologous system to study the function of the MarR protein CbdbA1625 in controlling expression of the *rdhA* gene *cbdbA1624* was developed. A heterologous *in vivo* system in *Escherichia coli* was established using a single-copy reporter-*lacZ* fusion comprising the intergenic region (IR) between *cbdbA1624* and the divergently transcribed *cbdbA1625*, encoding a MarR-type regulator. We analysed the activity of the promoter of *cbdbA1624* and that of *cbdbA1625*. Both promoters were functional in *E. coli* MC4100. The effect of multicopy *cbdbA1625* on expression of both reporter-*lacZ* fusions was analysed. The MarR-type regulator had a negative effect on expression of both promoters. This work demonstrates that heterologous expression systems provide a powerful approach to dissect the transcriptional regulation of *rdh* gene expression.

Acknowledgement: This work is supported by the DFG (research unit FOR1530)
[1] Kube *et al.*(2005) Genome sequence of the chlorinated compound-respiring bacterium *Dehalococcoides* species strain CBDB1. Nat Biotechnol 23, p.1269-1273.

OTP022

4-Sulfoacetophenone Baeyer-Villiger-type Monooxygenase and 4-Sulfophenylacetate Esterase in *Comamonas testosteroni* KF-1

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The xenobiotic laundry surfactant Linear Alkylbenzene Sulfonate (LAS) (3 x 10^6 tons per year worldwide, [*1*]) is completely degraded by heterotrophic bacterial communities [*2*]. However, until now, no information on the enzymes and genes involved has been established.
3-(4-Sulfophenyl)butyrate (3-C4-SPC) is a biodegradation intermediate of LAS, and 3-C4-SPC is mineralized by *Comamonas testosteroni* KF-1 [*3, 4*] through a pathway that involves 4-sulfoacetophenone (SAP) and an inducible Baeyer-Villiger-type monooxygenase (BVMO) to yield 4-sulfophenylacetate (SPAc) from SAP. The hydrolysis of SPAc to 4-sulfophenol (SP) and acetate is catalysed by an esterase. This SPAc-esterase was purified to homogeneity and the corresponding gene in *C. testosteroni* KF-1 identified by peptide-mass fingerprinting. A predicted BVMO gene was located directly upstream to the SPAc-esterase gene. This candidate gene was over expressed in *Escherichia coli* and purified. The recombinant enzyme catalyzed the NADPH-dependent oxygenation of SAP to SPAc, which was hydrolyzed after the addition of purified SPAc-esterase, yielding SP and acetate. Thus, the first two genes and enzymes involved in the complete degradation pathway for LAS have been identified and characterized.
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OTP023

Use of transcription factors to visualize small-molecules at the single cell level, and application for metabolic engineering

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Successful mutant development in microbial biotechnology relies on random mutations and combinatorial approaches. A current limitation is the subsequent screening of bacterial populations for cells with increased production properties. We developed sensors to quantify metabolites within a single cell. Together with FACS this enables the isolation of single producer cells from large mutant libraries. The system is based on a transcriptional regulator and its target gene fused to eyfp. Since transcriptional regulators exist which naturally sense numerous small-molecules, our technology enables a various new applications.
As one example we use the transcriptional regulator LysG of C. glutamicum sensing basic amino acids. Introducing the sensor pSenLys in a C. glutamicum mutant producing L-lysine or L-arginine resulted in strong fluorescent cells, which was not the case with controls. The key enzyme of L-arginine synthesis is the *argB* encoded acetylglutamatkinase which is inhibited in its activity by L-arginine. A plasmid-encoded *argB* mutant library was generated via epPCR and introduced into C.glutamicum carrying pSenLys. Applying FACS selection, sequencing and acetylglutamatkinase activity determination 16 *argB* alleles were isolated carrying 22 different mutations. Whereas wild type *argB* is inactive at 0.5 mM L-arginine, mutant alleles were selected which retained full activity at 4 mM L-arginine.
As another example we treated the wild type of C.glutamicum carrying pSenLys with N-methyl-N′-nitro-N-nitrosoguanidine. Out of 6.5 x 10^6 cells 270 cells were selected, of which 225 accumulated 3-38 mM L-lysine. Targeted sequencing identified 13 new chromosomal mutations in the known targets *lysC* and *hom*. From 10 mutants with no mutation in known targets the entire genome was sequenced using Illumina HiSeq 2000 technology. A *murE* mutation was identified which when introduced into existing L-lysine producers improved the L-lysine titers significantly

OTP024

Correlations between process parameters and the microcosm of biogas fermenters

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The influence of the process parameters hydraulic retention time (HRT), organic loading rate (OLR), substrate and temperature upon bacterial diversity was analyzed in automated fermenters. Therefore, a mesophilic (41°C) and thermophilic (55 and 60°C) anaerobic fermentation of beet silage as model substrate for renewable biomass was monitored by the amplified ‘‘ribosomal DNA’’ restriction analysis (ARDRA).
Surprisingly, a predominant population of hydrogen utilizing *Euryarchaeota* (represented by *Methanobacteriales*, *Methanomicrobiales*) was observed under all operating modes. The acetotrophic *Methanosaeta* ssp. and *Methanosarcina* spp. played apparently only a minor role among the operational taxonomic units (OTUs) found. Under thermophilic conditions *Methanosaeta* spp. could even not be detected.
This contradicts to common models for anaerobic digestion processes. An important finding was that under thermophilic conditions a change in temperature from 60 °C to 55 °C and back to 60 °C again was an important parameter to impact reversibly the morphological diversity of methanogenic Euryarchaeota. They changed from a mixture of methanogenic cocci and rods to an exclusive appearance of rods and vice versa. Under mesophilic conditions the temperature was held constant and variations of the hydraulic retention time (HRT) influenced remarkably the diversity of methanogens. Long HRTs (e.g. 37 days) kept the level of methanogenic species richness low, while quickly decreased HRTs (e.g. 8 days) induced a higher diversity and similar diversity patterns, respectively.
This study also revealed that the population dynamics, the species richness and diversity of hydrolytic and fermentative *Bacteria* was higher compared to the diversity of methanogenic *Archaea*.Under mesophilic and thermophilic fermentation temperatures, most of the detected OTUs could be assigned to the Phyla *Firmicute*, *Bacteroidetes* and *Proteobacteria*,while *Chloroflexi* appear to play an important but yet unknown role during a mesophilic biogas process with high nutrient levels of renewable biomass like beets. Astonishingly, only single bacterial phyla could be impacted. One explanation of this phenomenon could be the functional redundancy of carbohydrate degraders. The presence of the taxa *Planctomycetes*, *Actinobacteria* and *Alcaligenaceae* was related to long HRTs and short OLRs, while the Phylum *Acidobacteria* was governed by short HRTs and high OLRs, respectively.

OTP025

Identification of Klebsiella pneumoniae’s strains isolated from « urine » as a human pathological product and evaluation of their antibiotic resistance

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Our study was about the biochemical identification of Klebsiella pneumoniae’s strains which were isolated from “urine” as a human pathological product, in addition to the evaluation of their sensibility to antibiotics. The results synthesized from this research have shown that: K.pneumoniae has the ability to produce “acetoin” from “pyruvic acid”, hence it is characterized by a positive Voges-Proskauer reaction. The results of “the antibiogram” have confirmed the efficiency of “colistin” as an antibiotic on our strains. We have also shown the production of BLSE enzymes (Beta Lactamases with Extended Spectrum) by some strains. In addition to this, we have tested the effect of “inoculum” on the resistance to “cefotaxim” and to the association “amoxicillin + clavulanic acid” and the results have shown a widening of the circle’s diameter surrounding the antibiotic’s disc after dilution, which explains a higher sensibility of strains to antibiotics. This experience of “inoculum’s effect” has shown us that from a lower inoculum (after dilution) results a higher sensibility.

OTP026

Isomer and enantioselective carbon stable isotope fractionation of hexachlorocyclohexane during aerobic biodegradation by *Spingobium spp*

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In biochemical processes the preferential reactivity of the lighter stable isotope over the heavier stable isotope results in enrichment of the heavier isotopes in the residual substrate and relative enrichment of the lighter isotope in the products. The isomer and enantioselective carbon stable isotope fractionation of organic contaminants such as hexachlorocyclohexane and its chiral isomers (α-HCH) may be used to assess their fate in the environment. The extent ofin situtransformation may therefore be inferred by using experimentally determined compound specific isotope fractionation factors during biotransformation by defined microbial cultures. In this study, carbon isotope fractionation factors were determined for the biotransformation of α and γ-HCH using two aerobic bacterial strains: *Sphingobium indicum* B90A and *Sphingobium japonicum* UT26. Batch culture biodegradation experiments were performed and the carbon isotope fractionation of γ-HCH degradation was quantified by the Rayleigh equation. The bulk enrichment factor for γ-HCH was highly similar (εC= -1.8) for both *S. japonicum* UT26 and *S. indicum* B90A, but less compared previously reported values for anaerobic HCH dechlorination (-3.9±0.6) [1]. Additionally, the carbon isotope fractionation for α-HCH and its enantiomers was quantified. Interestingly, carbon isotope fractionation of α-HCH by *S. japonicum* was in a similar range to γ-HCH; for *S. indicum* fractionation was about 3 fold higher. Similarly, preliminary investigation showed that fractionation of α-HCH enantiomers was corresponding to the bulk isotope fractionation of α-HCH. The differences in fractionation may be due to the presence and activity of the different dehalogenases (Lin) in these organisms. Therefore, although a qualitative assessment of biodegradation of HCHin situmay be possible, a quantitative assessment requires further investigations.
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OTP027

The ability of Iranian traditional dairy bacterial strains to detoxification of Aflatoxin B1

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Introduction: Aflatoxins such as Aflatoxin B1 (AFB1) are highly toxic, mutagenic, teratogenic and carcinogenic compounds produced by some species ofAspergillus.They are found in many foods and feeds and considered as a major public health problem especially in developing countries. This study was conducted investigate the AFB1 detoxification ability of 60 probiotic bacteria isolated from Iranian traditional dairy products.
Method: A working solution of 5 µg/ml of AFB1 was prepared in phosphate-buffered saline (PBS, pH 7.3). Bacterial suspension was prepared by culturing the strains in MRS broth at 37°C for 20h. These

bacteria were resistant to acid, bile and digestive enzymes and were shown to lower the cholesterol levels in mice model. Bacterial cultures were centrifuged and the pellets were washed (3 times) and were suspended in in AFB1 solution with final concentration of 1-1.5×10^{10} CFU/ml. the bacterial solutions were incubated for 2 h at 37°C. The cell free supernatants samples were analyzed with a reverse phase high-performance liquid chromatography (HPLC) as well as the Enzyme Linked Immunosorbent Assay (ELISA).
Results: The results showed that the AFB1 binding capacity of strains was strain dependent. The strains were observed to possess variable AFB2-binding ability in the range from 8 to 63%. Most efficient binding of AFB1 was observed byL.plantarumTD14, andL.caseiTD15. The differences in the binding activities of AFB1 between the strains showed statistical significance ($p>0.05$). Our results indicated the protective ability of these indigenous probiotic strains against AFB1as well as their beneficial health effects. It is well documented that the AFB1 detoxification by these bacteria involves sequestration by binding the toxin to the bacterial cell wall. These findings suggest that certain novel probiotic bacteria isolated form Iranian traditional dairy products with high aflatoxin binding capacity could be selected for detoxification of foods.

OTP028

Identification of the protoporphyrinogen IX oxidase in *Pseudomonas aeruginosa*

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Heme is an important tetrapyrrole because of its function as a cofactor in several proteins which are linked to fundamental biological processes like respiration, photosynthesis, the metabolism and transport of oxygen (Layer *et al*, 2010). The biosynthesis of heme is a well studied process, nevertheless there are bacteria which obviously lack one or more of the known enzymes for this pathway and are still able to synthesize heme. For at least three steps during the heme formation it is known that there exist other enzymes responsible for catalysis (Boynton *et al*, 2011). One of these enzymes is the protoporphyrinogen IX oxidase (PPO). PPO oxidizes protoporphyrinogen IX to protoporphyrin IX which is the penultimate step in the heme biosynthetic pathway (Layer *et al*, 2010). Until today no known PPO-gene has been identified for *Pseudomonas aeruginosa*. Our approach was to isolate the oxygen-dependent PPO-gene from *P. aeruginosa* by complementation of an *Escherichia coli* PPO mutant with a *P. aeruginosa* ATCC 17933 gene library. UV/Vis and fluorescence spectra were recorded *via* high pressure liquid chromatography to measure the level of heme in apparently positive clones. Complementary genes from clones with high heme levels were sequenced. To investigate so obtained putative PPO we will knock out these genes in *P. aeruginosa* and complement the phenotype with the *E. coli* PPO (*hemG*). Furthermore, we will overproduce the putative *P. aeruginosa* PPO for their biochemical characterization.

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OTP029

Isolation of *Streptomyces* integrative chromosomal elements by rolling-circle amplification

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The order Actinomycetales consists of high G+C Gram-positive bacteria of which many species form a branching mycelium by apical tip extension. Actinomycetes, in particular the genus *Streptomyces*, are the most important source of biologically active microbial products, including antibiotics. As antibiotic producers, the actinomycetes represent the natural reservoir of resistance genes that are transferred to other bacteria by horizontal gene transfer (HGT).
The availability of genomic sequences of many actinomycetes revealed the presence of multiple integrative chromosomal elements (ICE). ICE´s are characterized by their prophage-like mode of maintenance as part of the chromosome, and their ability to excise, to promote their transfer to a new host, and to integrate into the host genome by site specific recombination. Since ICE´s normally disintegrate only prior to conjugation, which is regulated by unknown factors, such elements are very difficult to isolate by alkaline lysis.
Here we show that it is possible to amplify novel ICE´s from different *Streptomyces* strains using random hexamer primers and the Phi29 DNA polymerase. Sequence analysis of subcloned DNA fragments allows the rapid characterization of the newly isolated *Streptomyces* ICE´s.
The presented work was done as part of the "Mikrobiologisches Großpraktikum/Biotechnologie ([1]) and a bachelor thesis ([2]).

OTP030

A highly efficient *Staphylococcus carnosus* mutant selection system based on suicidal bacteriocin activation

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Strains from various staphylococcal species produce bacteriocin peptides, which are thought to play important roles in bacterial competition and offer interesting biotechnological avenues. Many bacteriocins are secreted as inactive pre-peptides with subsequent activation by specific proteolytic cleavage. By deletion of the protease gene gdmP in *Staphylococcus gallinarum* Tü3928, which produces of the highly active lanthionine-containing bacteriocin gallidermin (lantibiotic), a strain was created producing inactive pre-gallidermin. On this basis a new suicidal mutant selection system in the food-grade bacterium *Staphylococcus carnosus* was developed. Whereas pre-gallidermin was inactive against *S. carnosus*, it exerted potent bactericidal activity toward GdmP-secreting *S. carnosus* strains. To take advantage of this effect *gdmP* was cloned in plasmid vectors used for random transposon mutagenesis or targeted allelic replacement of chromosomal genes. Both mutagenesis strategies rely on rare recombination events and it has remained difficult and laborious to identify mutants among a vast majority of bacterial clones that still contain the delivery vectors. The *gdmP*-expressing plasmids pGS1 and pGS2 enabled very fast, easy, and reliable identification of transposon or gene replacement mutants, respectively. Mutant selection in the presence of pre-gallidermin caused suicidal inactivation of all clones that had retained the plasmids and allowed only growth of plasmid cured mutants. Efficiency of mutant identification was several magnitudes higher compared to standard screening for the absence of plasmid-encoded antibiotic resistance markers and reached 100% specificity. Thus, the new pre-gallidermin based mutant selection system represents a substantial improvement of staphylococcal mutagenesis methodology.

OTP031

Reductive dechlorination in *Desulfitobacterium hafniense* Y51: Impact of vitamin B_{12} o*n pceA* gene stability and expression

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Desulfitobacterium hafniense Y51 is a strictly anaerobic, gram-positive bacterium, which is able to grow with aliphatic chlorinated compounds, such as tetrachloroethene (PCE), as terminal electron acceptors. PCE is reductively dechlorinated to *cis*-1,2-dichloroethene. The key enzyme is the PCE reductive dehalogenase, a corrinoid cofactor and iron-sulfur cluster containing protein. All enzymes required for *de novo* corrinoid cofactor biosynthesis are encoded in the genome of *D. hafniense* Y51 (1). The gene encoding the PCE reductive dehalogenase, *pceA,* is organized in the *pceABCT* gene cluster. The cluster is flanked by two almost identical insertion sequences including transposase genes. The excision of the *pce* gene cluster from the genome of *D. hafniense* Y51 can occur (2).
In this study we investigated the impact of vitamin B_{12} added to the medium on the transposition of the *pceA* gene. In the presence of the growth substrate PCE the *pceA* gene remains stable in *D. hafniense* Y51 genome whether or not vitamin B_{12} was added to the culture. When cells were cultivated on fumarate instead of PCE and vitamin B_{12} was omitted from the medium, the number of *pceA* genes per culture decreased rapidly. Interestingly, this effect is strongly delayed when external vitamin B_{12} was provided (long-term effect).
To acquire the data presented here, cells repeatedly grown on the different media compositions were analysed for the number of *pceA* genes using quantitative PCR (qPCR), for the *pceA* transcript level using reverse transcription quantitative PCR (RT-qPCR), for the PceA enzyme activity and the amount of PceA protein using specific antibodies. In parallel, the expression of vitamin B_{12} biosynthesis genes was examined. Based on the results of this survey a positive effect of vitamin B_{12} on the *pceA* gene stability and expression in *D. hafniense* Y51 is discussed.

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OTP032

TrxR system - A new target in the fight against *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis (Mtb)depends on an efficient anti-oxidative system during infection. To maintain the survival, *Mtb* relies on the Thioredoxin Reductase (TrxR) system, because it lacks a glutathione

system. Even though, eukaryotes obtain TrxR system as well, the similarity is very low, therefore TrxR systems can be targeted to treat tuberculosis.
After an *in silico* high throughput screening for Trx-inhibitors, four different low mass scaffolds were identified. *In vitro* testing of compounds relying on those scaffolds was performed at recombinant expressed *Mtb* TrxR. The auspicious substances were tested in liquid *Mtb* cultures with MGIT 960 system (Becton Dickinson). We identified several substances that showed bacteriostatic effects on *Mtb* at μM concentrations.
We could show that by attacking the TrxR-system *in vitro* mycobacterial growth can be arrested.

OTP033

Nitrous oxide reductase with a unique [4Cu:2S] centre from denitrifying *Pseudomonas stutzeri*

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The genera *Pseudomonas* and *Paracoccus* include the most commonly isolated denitrifying bacteria from soils and aquatic sediments and may represent the most active denitrifiers in natural environments[3]. Denitrification is the dissimilatory reduction from the ionic oxides (nitrate and nitrite) to the gaseous oxides nitric oxide and nitrous oxide. The subsequent two-electron reduction of nitrous oxide to dinitrogen is the final step in the denitrification process[4]. Nitrous oxide is involved in atmospheric reactions and its accumulation in the stratosphere leads to depletion of ozone.
Nitrous oxide reductase, NosZ, is a dimeric multi-copper protein, with 638 residues per subunit (74 kDa) and the reported copper content depends on the purification strategy. Because of the high sensitivity of the enzyme toward dioxygen, the clusters of the soluble periplasmic enzyme degrade and it therefore loses its activity under aerobic conditions. In literature, several different forms were described, that can be distinguished by their typical absorption and EPR spectra. The active purple form of the enzyme carries the well-characterized mixed-valent binuclear Cu_A centre and the tetranuclear Cu_Z site, that was first described as a unique [4Cu:2S] centre[1] for *Pseudomonas stutzeri*, instead of the [4Cu:S] cluster[4] found previously. This newly described cluster was observed after the isolation and crystallization under the exclusion of dioxygen[2]. In nitrous oxide reductase the substrate N_2O is bound between the two copper centres, it is activated by side-on binding at Cu_Z, so that then electrons can be transferred directly from Cu_A to N_2O. Several accessory proteins were identified for the biogenesis of active N_2O reductase, with predicted functions as Cu chaperones or ABC transporters. To date the exact steps of cluster biogenesis and the mechanistic details of N_2O reduction are still unknown.

OTP034

Key enzymes of fuel oxygenate ether degradation

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The extensive use of methyl-*tert*-butyl and *tert*-amyl methyl ether (MTBE and TAME, respectively) as gasoline additives has resulted in persistent groundwater contamination due to their recalcitrance against microbial attack. However, we were able to isolate the bacterial strain *Aquincola tertiaricarbonis* L108 from an MTBE-contaminated aquifer (Leuna, Germany) which can grow well on all kinds of fuel oxygenate ethers as single source of carbon and energy [1]. We have now elucidated the underlying degradation pathways by generating gene knockouts specifically affecting expression of key enzymatic steps. In addition, central metabolites of ether catabolism were identified. Initial degradation proceeds via specific hydroxylation by the EthABCD monooxygenase system resulting in the formation of *tert*-butyl or *tert*-amyl alcohol (TBA or TAA). Degradation of the latter is mainly catalyzed by the monooxygenase MdpJ. TBA is hydroxylated to 2-methylpropan-1,2-diol, while TAA is desaturated to the hemiterpene 2-methyl-3-buten-2-ol. In a side reaction, TBA and TAA are dehydrated to the corresponding alkenes, i. e. isobutene and isoamylene isomers, by a not yet characterized enzymatic step [2]. The 2-methylpropan-1,2-diol is oxidized further to 2-hydroxyisobutyric acid, which is activated to the corresponding CoA ester and isomerized to the common metabolite 3-hydroxybutyryl-CoA by a specific cobalamin-dependent acyl-CoA mutase [1]. 2-methyl-3-buten-2-ol, on the other hand, is degraded via a hemiterpenic primary alcohol and the corresponding aldehyde and carboxylic acid, linking TAA degradation with the biotin-dependent catabolism of the amino acid leucine [3].
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F. Schäfer, L. Muzica, J. Schuster, N. Treuter, M. Rosell, H. Harms, R.H. Müller and T. Rohwerder, Appl. Environ. Microbiol.**77** (2011), p. 5981.

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OTP035

Marinobacter adhaerens hp15 is required for aggregation of the diatom, *Thalassiosira weissflogii*

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Aggregation of diatoms is an important process in marine ecosystems leading to the settling of particulate organic carbon predominantly in the form of marine snow. Exudation products of phytoplankton form transparent exopolymer particles (TEP), which act as adhesives for particle aggregation. Heterotrophic bacteria interacting with phytoplankton may influence TEP formation and phytoplankton aggregation. This bacterial impact has not been explored in detail. We hypothesized that bacteria attaching to Thalassiosira weissflogii might interact in a yet-to-be determined manner, which could impact TEP formation and aggregate abundance. The role of individual T. weissflogii-attaching and free-living new bacterial isolates for TEP production and diatom aggregation was investigated in vitro. T. weissflogii did not aggregate in axenic culture, and striking differences in aggregation dynamics and TEP abundance were observed when diatom cultures were inoculated with either diatom-attaching, i.e. Marinobacter adhaerens HP15, or free-living bacteria. The data indicated that free-living bacteria may not influence aggregation whereas bacteria such as M. adhaerens HP15 may increase aggregate formation. Interestingly, photosynthetically inactivated T. weissflogii cells did not aggregate regardless of the presence of bacteria. Comparison of aggregate formation, TEP production, aggregate sinking velocity, and solid hydrated density revealed remarkable differences. Both, photosynthetically active T. weissflogii and specific diatom-attaching bacteria were required for aggregation. It was concluded that interactions between heterotrophic bacteria and diatoms increased aggregate formation and particle sinking and thus may enhance the efficiency of the biological pump. M. adhaerens HP15 has become a genetically accessible model organism. Successful site-directed and transposon mutageneses, expression or reporter genes, and full access to the genome sequence of HP15 made this organism an ideal model strain to conduct the molecular dissection of the diatom-bacteria interaction at the cell-to-cell level.

OTP036

Will be presented as OTV032!

OTP037

Carbon stable-isotope fractionation of brominated ethenes by *Sulfurospirillum multivorans*

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Microbial dehalogenation has been investigated as a viable remediation strategy for contaminated field sites, as several bacterial species have been linked with biotransformation processes including reductive dehalogenation. Compound specific isotope analysis (CSIA) may be an effective tool for monitoring reductive dehalogenation activity in the environment if distinct fractionation patterns emerge during biodegradation studies in the laboratory. While the biodegradation potential of chlorinated ethenes has been extensively characterized, very little is known as regards biotransformation of brominated ethenes. However, certain bacterial strains, including *Sulfurospirillum multivorans* and *Desulfitobacterium* sp. strain PCE-S, which are capable of reductive dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE) to 1,2-dichloroethene (1,2-DCE), have also been shown to effectively debrominate tribromoethene (TBE) and 1,2-dibromoethene (1,2-DBE) under similar conditions [1].
Carbon stable-isotope fractionation had previously been determined during reductive dechlorination of PCE and TCE by *S. multivorans* and *Desulfitobacterium* sp. strain PCE-S [2], but had not been tested for the corresponding brominated compounds. This study aims to investigate the carbon-isotope fractionation of TBE and 1,2-DBE during reductive debromination by crude extracts of *S. multivorans*, and to evaluate these results against those for their chlorinated analogs. Preliminary results show brominated ethene fractionation patterns as similar to those for chlorinated ethenes by each strain, but to a lesser extent. In the case of TBE, fractionation was nearly negligible, contrasting with significant fractionation observed for TCE. However, fractionation observed during reductive debromination of 1,2-DBE, while less than that observed for 1,2-DCE, is significant, and suggests the potential use of CSIA for *in situ* assessments of reductive debromination. To further explore this potential, more studies are required to investigate fractionation occurring with other strains and likewise, with chlorinated ethenes.

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2. Nijenhuis, I., Andert, J., Beck, K., Kästner, M., Diekert, G., and H. H. Richnow. 2005. Stable isotope fractionation of tetrachloroethene during reductive dechlorination by *Sulfurospirillum multivorans* and *Desulfitobacterium* sp. strain PCE-S and abiotic reactions with cyanocobalamin. Appl. Environ. Microb. 71 (7), 3413-3419

OTP038

Screening halophilic and halotolerant bacteria from saline soil, mud, brine and salt sediments of Urmia lake in Iran

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Hypersaline lakes, with salinity ranges at or near saturation are extreme environments; yet, they often maintain remarkably high microbial cell densities and are biologically very productive ecosystems. To adapt to saline conditions, bacteria have developed various strategies to maintain cell structure and function. Studies of such bacteria are of great importance, as they may produce compounds of industrial interest. We employed culture-dependent techniques to study microbial diversity in Urmia Lake , a unique hypersaline lake (24.6% salinity) in northwest Iran. The samples were collected in November 2010 into sterile bottles and stored in ice boxes in the laboratory ,pH, moisture content, and Na+ , K+ , Ca2+ , Mg2+ , and Cl– content of the salt and sediment samples were measured according to standard methods. Screening bacteria from saline soil, mud, brine and salt sediments of Urmia lake led to the isolation of 280 moderately halophilic and 40 extremely halophilic bacteria among which there were 191 gram-positive rods, 99 gram- negative rods and 30 gram-positive cocci. PCR Amplification of 16S rDNA of isolates was carried out by using universal primers and products were sequenced commercially.These gene sequences were compared with other gene sequences in the GenBank databases to find the closely related sequences. Most of the isolates belonged to different species of genus Bacillus.

OTP039

Generating mutated variants of the unique 5-chloromuconolactone dehalogenase from *Rhodococcus opacus* 1CP and their comparison with the wildtype enzyme to elucidate catalytic relevant residues

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5-Chloromuconolactone dehalogenase ClcF plays an unique role in 3-chlorocatechol degradation by *R. opacus* 1CP. The variant of a so called modified *ortho*-cleavage pathway in that actinobacterium differs from the one typically found in proteobacteria by the inability of chloromuconate cycloisomerase ClcB2 to convert 2-chloro-*cis*,*cis*-muconate into *trans*-dienelactone. Instead, ClcB2 behaves like a muconate cycloisomerase catalyzing cyclization of 2-chloro-*cis*,*cis*-muconate to 5-chloromuconolactone. Further dechlorination to *cis*-dienelactone is performed by ClcF an enzyme showing high similarity to (methyl)muconolactone isomerases. Although these enzymes are typically involved in (methyl)catechol degradation their biochemical ability to catalyze dechlorination of chloromuconolactones has been recently reported.
As a first step to elucidate the mechanism of dechlorination as well as to identify residues, relevant for activity, mutational analysis of recombinant ClcF was made. Properties of variants were compared to wildtype ClcF as well as to muconolactone isomerase MLI and methylmuconolactone isomerase MMLI from (methyl)catechol-degrading *Cupriavidus necator* JMP134 in respect of changes in product formation (*cis*-/*trans*-dienelactone), kinetic parameters, and the ability to convert muconolactone. Using an *E. coli* / pET expression system and a three-step purification procedure turned out to be a well suited strategy to obtain recombinant proteins in high purity. A considerable extent of specialization of ClcF for its new physiological function in strain 1CP is indicated by an extremely low activity of that enzyme to convert muconolactone into 3-oxoadipate enollactone which represents the original function of (methyl)muconolactone isomerases. A similar picture was obtained by comparison of specificity constants towards 5-chloromuconolactone of ClcF (1.4 μM^{-1} s^{-1}), MLI (0.6 μM^{-1} s^{-1}), and MMLI (0.06 μM^{-1} s^{-1}).

OTP040

Identification of amino acids involved in substrate binding of PHB depolymerase PhaZ7 of *Paucimonas lemoignei*

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The extracellular PHB depolymerase PhaZ7 of *P. lemoignei* is unique among extracellular PHB depolymerases due to its specificity for amorphous native PHB granules (nPHB). The structure of PhaZ7 was solved first at 1.9 Å [1] and recently at 1.4 Å [2]. PhaZ7 is a single-domain globular protein with an α/β hydrolase fold and a catalytic triad consisting of S136, E242, and H306. Analysis of PhaZ7 structure showed a high similarity to lipase LipA of *Bacillus subtilis* except for the presence of an additional domain in PhaZ7 that is absent in LipA. This lid-like domain contained many hydrophobic amino acid residues suggesting their possible involvement in nPHB binding. Since the PhaZ7 structure has no accessible substrate entry to the catalytic site we suggest that conformational changes must take place upon substrate binding. The effects of mutations of selected hydrophobic amino acids of the PhaZ7 lid-like domain on activity and nPHB binding ability were investigated. Our results showed that mutations of Y105, Y176, Y189, Y190 and W207 to alanine or glutamate resulted in reduced nPHB depolymerase activity. Interestingly, a lag-phase of several minutes in the depolymerase reaction was observed before maximal activity was determined. Binding assays with nPHB revealed a reduced binding ability of these PhaZ7 muteins compared with wild type PhaZ7. The structure of Y105D and Y190D muteins were determined and revealed changes in the 280-290 region and in the 248-251 region. Recently, the structure of inactive PhaZ7 S136A mutein bound to 3-hydroxybutyrate (3-HB) trimer has also been determined. It showed that 3-HB trimer is bound to a groove surrounded by Y189/Y190, Y105 and Y176. This result is consistent with our mutagenesis results. Additionally, similar to the structure of the Y105D and Y190D muteins, the 280-295 region and the 248-253 region of S136A mutein bound to 3-HB trimer were missing indicating some flexibility of these regions. Hence, our hypothesis that hydrophobic amino acid residues of the PhaZ7 lid-like domain are involved in substrate binding and that conformational changes upon substrate binding occur was confirmed. Our results afford new insights into the mechanism of biopolymer binding to PHB depolymerases and enzymatic PHB hydrolyis.

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OTP041

Genome-guided analysis of physiological and morphological traits of the metabolically versatile fermentative acetate oxidizer *Thermacetogenium phaeum*

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Fermentative conversion of acetate to CO2 and hydrogen becomes possible if the hydrogen partial pressure is kept low by a methanogenic partner, but the energy gained from this process is very low. This relationship is called syntrophy.Thermacetogenium phaeum, isolated from a thermophillic anerobic methanogenic reactor, is able to grow on various substrates to form acetate as sole product, and in coculture with a methanogenic bacterium,Thermacetogenium phaeumis able to grow on acetate. It was shown previously that the Wood-Ljungdahl pathway is used in both modes of living, but the mechanism of energy conservation is unknown.
To extend our knowledge on the biochemistry and physiology of this interesting organism, we completely sequenced the genome ofThermacetogenium phaeum.The strain has one circular chromosome of the size of 2.93 Mb; the G+C content of the DNA is 53.88mol%. The manual annotation of the 3215 CDS encoded by the genome gave a deeper insight into the physiology of the organism.
All genes necessary for the Wood-Ljungdahl pathway were found but in comparison to the H^+-dependent acetogen Moorella thermoacetica and the Na^+-dependent acetogen Acetobacterium woodii no indications of cytochromes, sodium dependence, or of RNF-complexes were found as potential energy conserving mechanisms. It was reported that Thermacetogenium phaeum is a sulfate reducing bacteria but neither the genome sequence nor physiological experiments could confirm this result. As a sign of heavy phage attack in the past a lot of CRISPR sequences are present in the genome, and also a complete prophage was found.

OTP042

Construction of Rubber Oxygenase A variants (RoxA), a diheme-dioxygenase from *Xanthomonas* sp. 35Y

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The extracellular diheme-dioxygenase RoxA (Rubber oxygenase A) from *Xanthomonas* sp. 35Y is able to cleave natural rubber, the primary product is ODTD (12-oxo-4,8-dimethyltrideca-4,8-diene-1-al) [1]. The cleavage mechanism of this reaction is unknown. Heterologous expression of RoxA in *Escherichia coli*, *Bacillus subtilis* or *Pseudomonas putida* was not successful, therefore an overexpression of RoxA from a broad-host range rhamnose inducible plasmid was established in its natural host strain *Xanthomonas* sp. 35Y [2]. However, it was not possible to obtain recombinant RoxA with either a strep-tag or his-tag at the C-terminus. The

reason for this was revealed in DNA hybridization experiments that showed an integration of the expression vector into the chromosomal *roxA* copy. This event restricted the tags from the rhamnose inducible *roxA* copy. These results led to a construction of a *Xanthomonas* sp. 35Y *roxA*-deletion mutant and a vector that allows site specific but *roxA* independent integration of a *roxA* copy into the *Xanthomonas* sp. 35Y chromosome. Now it is possible to construct various RoxA muteins to investigate the reaction mechanism of RoxA. Relevant residues for potentially interesting mutation sites can be selected due to the similarity between RoxA and several well characterizied bacterial cytochrome-*c* peroxidases (CCPs), for example from *Pseudomonas aeruginosa* and *Nitrosomonas europaea*. Analogies were found in the distance and arrangement of the two heme centers as well as in some conserved residues. Despite the similarity of RoxA to CCPs RoxA has no peroxidase activity [3]. To simplify the purification of RoxA muteins, a strep-tag was added either to the C- or N-terminus. It turned out that only the N-terminal RoxA-strep-tag variant is stable. Unfortunately, a purification with the tag was not successful. Apparently, the tag is not completely accessible. Further experiments will aim at mutein construction and characterisation of these muteins to get a better understanding of the reaction mechanism of RoxA.

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OTP043

Stable isotope fractionation of monochlorobenzene during aerobic degradation by *Pseudomonas fluorescens*

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Monochlorobenzene (MCB) is a frequently detected groundwater contaminant due to its widespread use as a solvent and pesticide. Because of its toxicity and persistence in aquifers MCB represents an environmental issue. Therefore, it is important to investigate its fate in the environment, including biotransformation processes. It has been shown that MCB can be transformed by bacteria under aerobic and anaerobic conditions. Under aerobic conditions MCB can be used as sole carbon and energy source for bacterial growth. The aerobic MCB degradation is initiated by a dioxygenase and leads to the formation of chlorocatechol intermediates which then undergo either an ortho- or meta-cleavage. For the characterisation and assessment of *in situ* biotransformation processes stable isotope fractionation investigations are a valuable tool. The extent of isotope fractionation depends on the reaction mechanism of initial bond cleavage. Thus, the investigation of stable isotope fractionation might be used to characterise the biochemical reaction and *in situ* biodegradation of an organic contaminant. In the present laboratory study, carbon stable isotope fractionation during aerobic MCB degradation by *Pseudomonas fluorescens* DSM 16274 was investigated. In contrast to different aerobic MCB degrading strains tested in a fractionation study of Kaschl et al. [1] *Pseudomonas fluorescens* DSM 16274 uses the meta-cleavage pathway to break down 3-chlorocatechol. The obtained enrichment factor for the reaction was, however, in the same range as the ones using the ortho-pathway supporting that the aerobic pathway initiated by a dioxygenase does not result in a significant carbon isotope fractionation. These results suggest that in oxic environments microbial MCB degradation can hardly be distinguished from abiotic attenuation processes. However, a differentiation between aerobic and anaerobic biotransformation processes is possible due to the significant carbon isotope fractionation related to MCB degradation under anaerobic conditions.

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OTP044

Interaction of *Listeria monocytogene*s with free-living amoebae

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Listeria monocytogenes is among the most important food-borne pathogens. Despite the fact that the virulence mechanisms ofL. monocytogenesare very well characterized, and the demonstration of the ubiquitous distribution of *L. monocytogenes* in the environment, our knowledge about putative environmental reservoir(s) of *L. monocytogenes* is still limited.
In this study we investigated the interaction of *L. monocytogenes* with free living amoebae of the genus *Acanthamoeba*. In the environment as well as in food-production environments (e.g. drinking water systems), *L. monocytogenesis* faced with predation by ubiquitous protozoa. Particularly acanthamoebae have been shown to be important as hosts and shelters for pathogenic bacteria in the environment. We therefore speculated that amoebae might also represent an environmental reservoir for *Listeria monocytogenes*.

To test the ability of *L. monocytogenes* to survive in amoebae, we developed an infection assay. Using this assay, we could show that *L. monocytogenes* can survive in acanthamoebae. Using confocal laser scanning microscopy, we could also show the presence of *L. monocytogenes* in amoeba trophozoites and cysts. This is particularly interesting as amoebal cysts are highly resistant to various environmental stresses such as disinfectants, desiccation, or nutrient deprivation. The presence of *L. monocytogenes* in amoebal cysts might thus allow the survival of adverse environmental conditions and represent one putative reservoir of Listeria in the environment as well as food-production environments.

OTP045

The TrpD2 protein family, a novel class of DNA repair enzymes?

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The TrpD2 proteins are uncharacterized homologues of the anthranilate phosphoribosyltransferase (TrpD), a homodimeric enzyme involved in tryptophan biosynthesis [1-2]. There are about 140 known TrpD2 family proteins that are widespread among Bacteria, but do not occur in Archaea. They share on average 17 percent sequence identity with TrpD, but do not catalyze the TrpD reaction. We have set out to elucidate the biological function of the TrpD2 group.
We have solved the crystal structure of YbiB, the *E. coli* representative of the TrpD2 group. It is very similar to the structure of TrpD, but exhibits a positively charged surface groove with arginine and lysine residues conserved throughout the whole TrpD2 group. The shape of the groove and the charge distribution suggested that YbiB might bind nucleic acids. Indeed, binding of single stranded DNA to YbiB and other TrpD2 proteins could be detected and quantified by electro mobility shift assays, fluorescence spectroscopy, fluorescence polarization, and surface plasmon resonance. The binding is characterized by a K_D value of 6 to 60 nM and shows no sequence specificity. Single stranded RNA is bound equally well, whereas the affinity for double stranded DNA is two orders of magnitude lower.
The *ybiB* gene forms a LexA-controlled operon together with a gene encoding a DNA helicase. This finding points to a possible involvement in the *E. coli* SOS response to DNA-damaging conditions. In support of this hypothesis, we could show that YbiB confers enhanced resistance against the mutagenic substance mitomycin C (MMC) *in vivo*. Our results suggest that the TrpD2 proteins represent a novel class of DNA repair enzymes that might recognize lesions such as interstrand crosslinks. The excision of damaged bases could be accomplished via phosphorolysis, since both the TrpD and TrpD2 enzymes are evolutionary linked to the class II nucleoside phosphorylases [3]. These enzymes cleave off the base from a nucleoside using inorganic phosphate. YbiB presumably has a phosphate binding site at its putative active center, which is located near the DNA binding groove. Proteomic data support our DNA repair hypothesis.

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OTP046

Microthrix parvicella and *Cloacamonas acidaminovorans*: Indicator organisms for foam formation in large-scale biogas plants?

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Anaerobic co-fermentation of sewage sludge and waste with the objective to produce biogas is of growing interest to generate renewable energy and to reduce greenhouse gas emissions. An anaerobic digester is still operated as a so called “black box” and process failures such as foam, over-acidification or floating layers occur in various plants. Changes in the microbial community during process failures could already be observed in laboratory-scale fermenters. However, the alteration in the microbial biocenosis during process failures in large-scale biogas plants is scarcely investigated.
In our studies the variances of the microbial community during a foam formation in a sewage sludge and grease fed biogas plant, consisting of four 8.000.000 litre biogas reactors, were analyzed. To compare the diversification in the microbial community, the partial 16S rDNA genes of the two microbial domains Bacteria and Archaea were analyzed by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and microorganisms were identified by sequence alignment. A relative quantification of possible indicator organisms was carried out

using real-time PCR. Activity measurements and analysis of spatial relationship are planned via fluorescencein situhybridization (FISH).
The molecular fingerprinting revealed an altered microbial biocenosis during a foam formation event and over a one-year period in the foaming-prone reactor. *Microthrix parvicella* and *Cloacamonas acidaminovorans* seemed to be directly connected to the foam formation. Higher cell numbers of these two organisms were detected in the foam. Real-time PCR measurements verified higher DNA amounts of *M. parvicella* in the foaming reactor and foam. Additionally, higher cell numbers of *M. parvicella* could be detected in the winter months possibly caused due to temperature sensitivity.
M. parvicella and *C. acidaminovorans* could act as indicator organisms for a starting foam formation in large-scale biogas plants. Finding a threshold DNA concentration of *M. parvicella* or *C. acidaminovorans* could serve as early-warning indicator to take countermeasures against a foam formation.

OTP047

Monomerization of the dimeric polyprenylglyceryl phosphate synthase PcrB by protein design results in a different substrate specificity

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The bacterial PcrB proteins show about 35% sequence identity to the archaeal geranylgeranylglyceryl phosphate synthases (GGGPS). PcrB has recently been shown to be a heptaprenylglyceryl phosphate synthase, which catalyzes the formation of an ether bond between *sn*-glycerol-1-phosphate (G1P) and heptaprenyl pyrophosphate (HepPP) [1-2]. The crystal structure of *Bacillus subtilis* PcrB reveals a G1P-binding site as well as a long hydrophobic groove similar to the geranylgeranyl pyrophosphate binding site of *Archaeoglobus fulgidus* GGGPS [3-4]. However, the "ruler" limiting the length of the polyprenyl pyrophosphate to 20 C-atoms in GGGPS is missing in PcrB, allowing the binding of HepPP which contains 35 C-atoms.
Both GGGPS and PcrB form homodimers. The subunit interface has been unambiguously determined for GGGPS, whereas the published contact between the two PcrB subunits [3] is implausible due to the relatively small buried surface area. We therefore decided to identify the native contact interface of PcrB and to study the impact of dimerization for protein stability and substrate specificity. Bioinformatic analysis predicted two alternative interfaces, one of them being identical to the GGGPS interface. In order to loosen the dimer, we introduced destabilizing amino acids individually into the two predicted interfaces. Monomerization was exclusively observed with mutations in the surface area that corresponds to the GGGPS interface. Furthermore, we incorporated the non-natural aminoacid p-azido-L-phenylalanine at specific sites into each potential interface using the method developed by Schultz and coworkers [5] to crosslink the protomers. The experiment confirmed that PcrB has the same contact interface like GGGPS. The stability of the monomerized variants was not severely affected. However, their substrate specificity was limited to shorter polyprenyl pyrophosphates (geranyl pyrophosphate, 10 C-atoms). This finding shows that dimerization of PcrB is a prerequisite to bind and process the native polyprenyl pyrophosphate substrate.

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OTP048

Phylogenetic relationships among bacteria described from algae: Distinct source of new taxa

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Bacteria are an inherent part of the physical environment of algae. Algae are key components of the aquatic environments and are substrates for millions of microorganisms waiting to be discovered. Recent investigations have shown that bacterial communities associated with algae are highly specific to their host. Worldwide, representatives of several new bacterial species and genera have been isolated from algae. We conducted a phylogenetic study based on 16S rRNA gene sequences available in GenBank of 101 bacterial species (only type strains) which have been described as new species and have been derived from eukaryotic macro- and micro-algal sources. We found a clear dominance of 6 major bacterial lineages. The major lineage corresponded to Bacteroidetes with 42 newly described bacterial species, followed by Proteobacteria (including Alpha- and Gammaproteobacteria) with 36 species. Firmicutes, Actinobacteria, Verrucomicrobia and Planctomycetes contributed to a lesser extent. Based on the information of the species descriptions, 32% of all new bacterial species were able to decompose macroalgal polysaccharides, especially the members of Bacteroidetes and Gammaproteobacteria. On the other hand, most of the bacteria described from marine microalgae grouped into the Alphaproteobacteria, indicating that some members of this group are well adapted to live in close association with phytoplankton. We confirmed algae as a distinct source for new bacterial taxa. Although such associations can be random or specific, they could be explained by evolutionary adaptations through metabolic pathways, niche specificity or mutualistic relationships. Those parameters might play an important role in algae-bacteria relationships in nature.

OTP049

Novel Octaheme Cytochromes c enzymes

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Multiheme Cytochromes c (MCC) are a diverse family of electron carriers and redox enzymes that play a central role in several metabolic pathways. Some MCC enzymes have been structurally characterized in the past and were found to contain conserved heme-packing motifs, although their primary structures are largely unrelated [1,2]. Interestingly, purified MCCs are able to convert more than one substrate. However these activities have to be interpreted carefully for the fact that not every measured in vitro activity has a compulsory physiological role.
The classical enzyme displaying a wide substrate versatility is NrfA, an ammonium-producing pentaheme cytochrome c nitrite reductase, that catalyses the six-electron reduction of nitrite to ammonia as the key reaction in respiratory nitrite ammonification. It is also able to convert hydroxylamine, nitric oxide, and sulfite [3,4]. Other already characterized MCCs belong to the family of Octaheme Cytochomes C (OCC), like octaheme cytochrome c nitrite reductase (Onr) [5], octaheme tetrathionate reductase (Otr)[6] or the hydroxylamine oxidoreductase (HAO) [7]. The latter is so far the only OCC known to function as an oxidase. This is mainly due to an unusual cross-link of a tyrosine with a heme meso carbon of the active-site heme.
Another so far uncharacterized class of OCC are the εHAO, found in some Epsilonproteobacteria, such as some Campylobacter species [8]. These organisms lack a NrfA homologue and yet are reported as nitrite ammonifiers. Although the enzymes clearly are related to 'classical' HAO, the active-site tyrosine residue is absent in εHAO. It has been hypothesized that this enzyme reduces nitrite to hydroxylamine but it might just as well perform nitrite reduction to ammonium, thereby functionally replacing NrfA.
To broaden our knowledge of MCCs we focus on the structural properties that lead to substrate versatility of MCCs. Therefore we use high-resolution X-ray crystallography in combination with in vitro activity assays.
As a first step we were able to purify two octaheme εHAO, from Campylobacter curvus and Campylobacter concisus and observed nitrite reductase activity which is indeed lower than NrfA activity but still high enough to play a physiological role.

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OTP050

Characterization of the potential heme chaperone HemW

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Modified tetrapyrroles are complex macrocycles and the most abundant pigments found in nature. They play a central role in electron transfer dependent energy generating processes such as photosynthesis and respiration. They further function as prosthetic groups for a variety of enzymes, including catalases, peroxidases, cytochromes of the P450 class and sensor molecules. Heme is a hydrophobic molecule and associates non-specifically with lipids and proteins in aqueous solution where it promotes peroxidations. Due to its hydrophobicity und toxicity, heme has to be transported to its target proteins by different mechanisms, e.g. transport by transmembrane proteins, heme binding proteins and heme chaperones.
We identified *E. coli* HemW as a potential heme-binding protein. To characterize the heme-binding *E. coli* HemW was overproduced, anaerobically purified and a gel permeation chromatography was performed. Upon heme supplementation HemW dimerizes.
First EPR spectra of *E. coli* HemW incubated with heme revealed an spectrum typical of an oxidized $[4Fe\text{-}4S]^{3+}$ cluster indicating electron

transfer from the cluster to heme. Supplementation of HemW with an EPR active Fe-Corrol revealed a 5x- and to a lesser extent 6x- coordinated heme, the latter being an unusual form of coordination for heme.
For further characterization of heme binding different spectroscopic methods will be used (Raman resonance, Mössbauer, MCD, ITC) with the determination of the involved amino acid residues, the function of the iron sulphur cluster and SAM.
To verify that HemW is truly a heme chaperon, heme-free Nitrate-Reductase and Cytochrome bd oxidase membrane vesicles will be tested for heme transfer.

OTP051

Insights into the ecological distributions of the widely distributed *Dehalococcoides*-related Chloroflexi in the marine subsurface

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Bacteria of the phylum Chloroflexi appear to be widely distributed and sometimes abundant in the marine subsurface. Most subsurface Chloroflexi form a distinct 'class level' clade that are affiliated with organohalide-respiring *Dehalococcoides* strains. Despite the apparent global ubiquity of these '*Dehalococcoides*-related Chloroflexi' (DRC), little is known about their specific distributions and/or functional properties. In this research, specific PCR primers targeting 16S rRNA genes of the DRC were designed and employed to study the distributions of DRC in various subsurface environments. The assay proved highly specific and enabled the detection of a diverse range of DRC, often revealing the co-existence of diverse DRC phylotypes even within single subsurface samples. Quantification of DRC in marine sediment cores from a collection of globally dispersed locations by real-time PCR suggests these bacteria are seemingly ubiquitous and establish highest numbers in the shallow subsurface (i.e., in the upper meters), yet survive and persist with burial. Pyrosequencing of DRC through various marine sediment cores enabled high coverage of DRC diversity and therefore enabled patterns of diversity through depth to be clearly distinguished. This approach also revealed shifts in sub-groups of DRC through depth and suggested different sub-groups within the DRC favor different biogeochemical conditions, and therefore these sub-groups likely utilize different modes of metabolism.

OTP052

Bacteria from the Baltic Sea involved in the degradation of terrestrial DOC

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Permafrost soils of the northern hemisphere store large amounts of terrigenous dissolved organic carbon (tDOC). Climate change is expected to result in a significantly increased transport of tDOC to marine habitats. In order to assess the role of increased tDOC mobilization for the global carbon budgets, the potential of tDOC degradation in the marine environment needs to be quantified. In the current study, key bacterial species involved in the degradation of tDOC in the Baltic Sea were studied. Because of its unique salinity gradient that ranges from nearly limnic to marine conditions and since it has been shown that the bacterial community changes consistently along this salinity gradient, the Baltic Sea represents a suitable model system to study tDOC degradation under different environmental conditions. Incubation experiments were performed in which Baltic Sea water was supplemented with fresh tDOC originating from the River Kalix (next to Överkalix, North Sweden). High throughput cultivation was used to recover relevant bacterial isolates through the MultiDrop technique during different stages of tDOC degradation. Six different growth media were designed that contain typical constituents of tDOC including a polymer mix and soluble and insoluble humic analogs. Changes in culturability were quantified through the most probable number technique. Community composition of culturable bacteria was assessed by DGGE-fingerprinting of 16S rRNA genes. First results reveal specific changes in the community composition of bacteria that lead to the dominance of different bacteria during the different stages of the tDOC degradation.

OTP053

Acetone activation by strictly anaerobic bacteria

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Degradation of acetone by strictly anaerobic bacteria is being investigated with the sulfate-reducing bacterium Desulfococcus biacutus. An initial ATP-dependent carboxylation reaction has been proposed in the activation of acetone for aerobic and facultative anaerobic bacteria. In both types of bacteria acetone is carboxylated to form acetoacetate as an intermediate. The mechanism proposed for those bacteria requires the investment of two ATP equivalents for the initial step in the activation of acetone. In the case of sulfate-reducing bacteria, this carboxylation reaction is less likely to occur. The extreme energy limitation of the degradation of acetone coupled to sulfate reduction would not allow the sulfate reducers to apply a carboxylation reaction as the initial step. Therefore, we assumed that sulfate-reducing bacteria use a different strategy in the activation of acetone which is less energy expensive. A carbonylation reaction was hypothesized for activation of acetone by D. biacutus. This carbonylation would lead to 3-hydroxybutyrate or to an aldehyde derivative. Preliminary studies of the proposed carbonylation suggest that this reaction could take place in the activation of acetone. The acetone degradation in cell suspension experiments with D. biacutusshowed a sulfate-reducing activity faster and higher in the presence of CO than in the presence of CO_2. Aldehyde dehydrogenase activity was detected specifically induced in cell extracts of acetone grown cells. This activity was enhanced by the presence of ammonium in the test. Two dimensional electrophoresis with extracts ofD. biacutus showed different induced proteins in acetone grown cells. MALDI-TOF-MS analysis of one of the acetone induced proteins resulted in an unknown protein.

OTP054

Overexpression and purification of membrane proteins from *Gluconobacter oxydans*

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Gluconobacter oxydans is a member of the Gram-negative Acetobacteraceae that performs rapid incomplete oxidation of many sugars, sugar acids, polyols and alcohols. This feature has been exploited in several biotechnological processes (e.g. production of vitamin C and the antidiabetic drug miglitol). The genome sequence of *G. oxydans* 621H is known and it was found to contain over 70 uncharacterized oxidoreductases. For industrial bioconversions, membrane-bound dehydrogenases are of major importance since the products are excreted into the medium to almost quantitative yields. However, the overexpression and purification of membrane-bound proteins is generally difficult and time consuming. The membrane-bound glucose dehydrogenase, encoded by *gox0265*, was expressed from the previously constructed plasmid pBBR1p452[1] in *G. oxydans* ΔhsdR in an attempt to improve the process of integral membrane protein purification. The vector pBBR1p452 was constructed for gene expression in *Gluconobacter* spp. and its promoter displayed moderate strength.[1] Additionally, a C-terminal StrepTag was incorporated into the expression construct. Membranes of the overexpression strain had a specific activiy of 15 U/mg with glucose, which was seven-fold higher in comparison to the control strain. The rate of oxygen consumption of these membranes was very high (1100 nmol ½ O_2 $min^{-1}mg^{-1}$) and about three-times higher in comparison to the control. Glucose dehydrogenase was successfully purified from the membranes by solubilisation with detergent and subsequent StrepTactin affinity chromatography. Purified mGDH had a specific activity of 150 U/mg using D-glucose as substrate. Lower activities were also found with D-allose (43 % of activity compared to D-glucose), D-xylose (11 % of activity compared to D-glucose), D-galactose (7 % of activity compared to D-glucose) and D-gulose (4 % of activity compared to D-glucose). The K_M for glucose was 3.4 mM and V_{max} was 156 U/mg. These results demonstrate, that the purification of active membrane proteins by StrepTactin affinity chromatography is possible and can be used for the characterization of novel dehydrogenases.

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OTP055

Inducible gene expression and protein production in *Methanosarcina mazei*

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The methanogenic archaeon *Methanosarcina mazei* (*Ms. mazei*) is able to utilize different growth substrates such as H_2/CO_2, acetate, methylamines, and methanol. Many enzymes involved in the complex pathways of methanogenesis have been analyzed by heterologous overproduction in *E. coli*. However, for many methanogenic proteins this was not successful due to unusual prosthetic groups that will not correctly assemble in *E. coli*. Hence, a method for homologous production of proteins in *Ms. mazei* is desirable.
As a first step towards the production of complex proteins, the simple reporter protein β-glucuronidase from *E. coli* was fused to the inducible promoter p1687 from *Ms. mazei* using the shuttle vector pWM321 [1]. In the *Ms. mazei* genome, the p1687 promoter is located upstream of the gene cluster *mtt1/ mtb1* that is transcribed during growth on trimethylamine but down-regulated by a factor of 200 when the cells grow on methanol [2].

When *Ms. mazei* pWM321-p1687-uidA utilized methanol as a substrate, β-glucuronidase activity was almost not detectable indicating a tight regulation of gene expression by the p1687 promoter. Induction by the addition of trimethylamine led to a strong increase of expression of the *uidA* gene and β-glucuronidase activity was monitored by the production of *p*-nitrophenol from *p*-nitrophenyl-β-D-glucuronide.
In summary, we describe the first inducible gene expression system in *Ms. mazei*. This will be used for the overproduction and characterization of proteins that cannot be produced in *E. coli* and other simple expression systems. This will be of particular interest for proteins that harbour complex prosthetic groups that are hardly found or absent in the domain *Bacteria*, e.g. tungsten enzymes.

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OTP056

Antibacterial investigation of *Artemisia campestris L* (Asteraceae)

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Artemisia campestris L. (Asteraceae) is folk Libyan medicinal, small aromatic perennial shrub that grow in North Africa and most of Europe. The grounded of aerial parts was extracted in soxhlet apparatus successively, each crude extract was subjected to antibacterial evaluation against human pathogenic bacteria,Staph.aureus, E.coli, Salmonella spp. And Ps. aeruginosa, by using agar cup-cut diffusion assay .The results reported that chloroform and methanolic extracts were effective againstStaph.aureus in which shown by MIC is 12.5mg⁄ml.

OTP057

Screening for thermostable cellulases for lignocellulosic biomass degradation

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Existing biorefineries for ethanol production mainly use starch-biomass such as wheat and corn. To avoid the usage of feed- and foodstuff, lignocellulosic biomass lately attained particular interest of research. Lignocellulosic material like wheat straw is a challenging substrate due to the compact, often crystalline structure of cellulose-, hemicellulose- and lignin-polymers. To obtain fermentable sugar-monomers from cellulose and hemicellulose by enzymatic degradation, the wheat straw has to be decomposed, e.g. by hydrothermal processes.
To discover novel thermostable cellulases for degradation of the cellulosic fraction, suitable environmental samples (T = 60-90°C, pH 5-7) were enriched by using cellulose as sole carbon source. The DNA of the cultured microbial consortia was isolated for metagenomic library construction. Subsequently, the gene library was screened for the presence of cellulase encoding genes by detection of endoglucanase, cellobiohydrolase and β-glucosidase activity using colorimetric activity assays. Additionally, for activity-based screening, metagenomic libraries were constructed, directly using isolated DNA from hot springs from the Azores without previous enrichment procedures. Furthermore a sequence-based screening approach was also applied using sequence data of a metagenome. By aligning the nucleotide sequences with known genes, potential cellulase-encoding open reading frames were identified.
The activity-based screening revealed genes encoding putative endoglucanases and β-glucosidases. The sequence-based analysis resulted in the detection of one gene encoding another putative endoglucanase. Further work will be performed to express the identified genes in a suitable host system such as *E. coli* and *P. pastoris*. The corresponding enzymes will be tested with regard to activity towards the cellulosic fraction of the decomposed wheat straw.

OTP058

Translational regulation in *Haloferax volcanii*

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Translational regulation is an important cellular mechanism for gene expression control and is present in all three domains of life. It enables the cell to answer very rapidly to changes in environmental conditions and is thus involved in cell survival, differentiation, stress adaptation and response to specific stimuli.

To gain a global overview of growth phase-dependent translational regulation translatome analyses were performed with *Haloferax volcanii* and *Halobacterium salinarum* (Lange et al., 2007). Polysome-bound mRNA was separated from free mRNA by sucrose gradient centrifugation and the two mRNA fractions were compared using DNA microarrays. Thereby it was revealed for the two species that 6% and 20%, respectively, of all genes showed growth phase-dependent differential translational regulation (Lange et al.,2007). In *H. volcanii* many transcripts were translated with under-average efficiency in exponential as well as stationary phase, indicating that their translation might be induced in response to a different stimulus. Therefore, currently translatome analyses are performed after the application of various stress conditions, e.g. high and low osmolarity, high and low temperature, oxidative stress, poor carbon sources.
It was also revealed that the 5´- and 3´-UTRs are necessary and sufficient to transfer translational control from native transcripts to a reporter transcript. The 5´-UTRs are apparently necessary to down-regulate constitutive translational initiation, while induction of translation is encoded in the 3´-UTRs (Brenneis and Soppa, 2009).
However, the molecular mechanism and involved proteins are still unknown. Therefore, the *H. volcanii* genome was searched for putative RNA-binding proteins. To gain insight into their function the respective genes for selected proteins were deleted and a conditional overexpression system was generated. Analysis of the deletion and overexpression mutants is currently under way.

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OTP059

Biocatalytical Cyclization of Citronellal

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Hopanoids stabilize the cytoplasm membrane of many bacteria similar to the function of sterols in eukarotes. Key enzyme of hopanoid biosynthesis is the squalene-hopene cyclase (SHC) which catalyzes the polycyclization reaction of squalene to the pentacyclic triterpene hopene - the precursor of all hopanoids. The SHC-catalyzed reaction is one of the most complex biochemical reactions and involves the formation of 5 ring structures, the alteration of 13 covalent bonds, and the formation of 9 stereo centers. Zymomonas mobilis- an important ethanol producing bacterium - harbours two SHC-encoding genes that were cloned and over-expressed in E. coli. Hopene-forming activity was confirmed for both SHCs. One of the SHCs was additionally able to cyclise the monoterpene citronellal to isopulegol. This finding is contrary to former results using the model SHC from Alicyclobacillus acidocaldarius[1, 2] and several other SHCs cloned from different organisms in this study. Isopulegol is used as a flavor in different products and is an important intermediate in the production of menthol. Our finding is remarkable because cyclization of mono-, sequi- and diterpenes normally requires activation of the linear precursor by diphosphate[3, 4]. Depending on the stereo-configuration of the substrate different isopulegol stereoisomers were formed. Cyclization of citronellal by SHC is the first example of an enzyme-catalyzed cyclization of a not-activated linear monoterpene.
Further work focussed on the optimization of the SHC-catalyzed cyclization of citronellal by mutagenesis of SHC active site amino acids. Several SHC muteins revealed a strong increase in isopulegol-forming activity. Some muteins were able to catalyze an almost complete conversion of citronellal to isopulegol (≥ 90%) compared to only 30% for the wild type enzyme. Interestingly, the stereo-configuration and the relative isomer composition of the product were altered in some muteins. An overview on the cyclization potential of wild type and mutant SHCs from different sources will be given.

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OTP060

Development of a novel system for the functional expression and screening of membrane proteins

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The heterologous expression of membrane proteins and enzymes using standard expression hosts as *E. coli* is often hampered by many different factors including low expression efficiencies, degradation of the product, product toxicity, insufficient protein folding or formation of inclusion

bodies. Hence, we started to develop a novel bacterial expression system for the synthesis of membrane proteins that is based on the phototsynthetic bacterium *Rhodobacter capsulatus*. Due to its unique physiological properties the photosynthetic bacterium *R. capsulatus* is particularly suited for the high-level expression of membrane bound enzymes in an active form: Phototrophic growth conditions induce an intracellular differentiation of the inner membrane, leading to the formation of membrane vesicles in *R. capsulatus*. The membrane vesicles in turn provide an intrinsically high protein folding and incorporation capacity.
In order to evaluate the optimal growth conditions for heterologous membrane protein expression we started to express two different membrane proteins, the bacteriorhodopsin from *Halobacterium salinarium* as well as the squalene epoxidase from *Stigmatella aurantiaca*, under phototrophic, non-phototrophic as well as shifted conditions. Furthermore, integration of the heterologous membrane proteins into the photosynthetic membrane vesicles was confirmed by protein localization studies. The novel *R. capsulatus* expression system will now be used to identify novel membrane bound monooxgenases from metagenomic libraries.

OTP061

Dehalococcoides sp. strain CBDB1 reductively dehalogenates bromobenzenes to benzene in a respiratory process

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Brominated aromatics have broad applications in industry as flame retardants and fumigants or as intermediates during the synthesis of dyes, agrochemicals, pharmaceuticals and herbicides. By now, many brominated compounds are widespread contaminants in the environment and are regarded as potentially harmful to humans and the environment. However, brominated aromatics are also released naturally, particularly in marine ecosystems by algae, polychaets, sponges and molluscs. The complete removal of all halogen substituents is a crucial step in the degradation process and for further mineralization of halogenated compounds. A bacterial group which is known for its ability to reductively dehalogenate a broad range of toxic chlorinated compounds such as chloroethenes, chlorobenzenes, chlorobiphenyls and dioxins is the genus of the *Dehalococcoides*.
In this study we investigated whether the pure *Dehalococcoides* sp. strain CBDB1 is able to dehalogenate brominated benzenes, which were chosen as 'model' molecules for other more complex brominated compounds from natural or anthropogenic sources. Cultivation of strain CBDB1 with 1,2,4-tribromobenzene, three different dibromobenzene congeners or monobromobenzene revealed that all tested bromobenzenes were reductively dehalogenated to benzene in a respiratory process. Growth yields of 1.8 x 10^{14} to 2.8 x 10^{14} cells per mol of bromide released were obtained. Additionally a newly designed methylviologen based enzyme activity test was established to assess enzyme activity towards bromobenzenes. Furthermore mass spectrometric analyses of reductive dehalogenases were carried out to gain deeper insight into expression patterns of reductive dehalogenases after cultivation with different bromobenzenes. Our findings indicate that the same enzymes are involved during bromobenzene reduction as during chlorobenzene reduction, and suggest that *Dehalococcoides* sp. strain CBDB1 can be used for remediation of brominated aromatic contaminants.

OTP062

BlueTox: A novel genetically encoded photosensitizer

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Fluorescent active dyes and proteins like the green fluorescent protein (GFP), isolated from the jellyfish *Aequorea victoria* and members of the GFP-like protein family generates reactive oxygen species (ROS) as a byproduct of its fluorescence activity [(1)]. Thereby, the amount of generated ROS is strongly dependent on the proteins structure [(2,3)]. One example for a high-level ROS-producing fluorescent protein is KillerRed, a derivate of the non-fluorescent chromoprotein anm2CP isolated from *Anthemedusae sp*. [(4)]. This photosensitizer enables the light-mediated directed inactivation of targeted cell-structures and/or whole cells by application of the chromophore-assisted-light-inactivation (CALI-) technique [(5)]. As an alternative to this red fluorescent photosensitizer we developed, on basis of a FMN-based-fluorescent-protein (FbFP) [(6)], the novel photosensitizer BlueTox. BlueTox harbors a LOV-domain (light, oxygen, voltage) that binds flavinmononucleotide (FMN) as fluorophore and shows the characteristic excitation and emission maxima at 450nm^{ex}/495nm^{em}, respectively.
We demonstrated the blue-light induced, ROS-mediated photosensitizing effect of BlueTox by heterologous expression of the photosensitizer in *Escherichia coli* and subsequent time-resolved irradiation studies. The results of our *in vivo* analyses revealed a significant correlation between decrease of the amount of living cells and irradiation time. Therefore, BlueTox is a powerful tool for light-mediated inactivation of bacteria with high spatio-temporal resolution.

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OTP063

Production of the liposomase in *Clostridium sporogenes* for the therapeutic use in tumor therapy

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Solid tumors and their environment possess certain features that are unique in the human body. The most striking one is oxygen deprivation. These regions offer obligate anaerobic bacteria, such as clostridia, optimal conditions for growth. However, the colonization of the tumors alone is not sufficient for a complete tumor regression (Ryan et al., 2006). By genetic modifications, these bacteria can function as vectors delivering therapeutic proteins or prodrug-converting enzymes to their targets resulting in a direct effect on the remaining tumor tissue.
In this project, the liposomase is used for this purpose. The liposomase is a protein originally isolated from *Clostridium novyi* that can destroy liposomes (Cheong et al., 2006). Liposomes are membranous vesicles which can function as carrier for anticancer drugs such as doxorubicin, as these vesicles specifically accumulate in tumor tissues. However, the drug release from the liposomes is very slow due to their chemical and physical stability (Gabizon et al., 2006). Therefore, a genetically engineered strain of *Clostridium sporogenes* producing this enzyme should greatly enhance drug delivery from liposomes. *C. sporogenes* is a proteolytic and spore-forming organism that proved to be an excellent colonizer of hypoxic tumor tissue (Brown and Liu, 2004). For the expression of the liposomase gene in this organism a protein expression system based on the T7 system was constructed. The resulting expression mutant of *C. sporogenes* should produce and secrete the liposomase in the surrounding medium in a sufficient concentration providing a more effective strategy in the fight against cancer.

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OTP064

ClubSub-P: cluster-based subcellular localization prediction for Gram-negative bacteria and archaea

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The subcellular localization (SCL) of proteins provides important clues to their function in a cell. In our efforts to predict useful vaccine targets against Gram-negative bacteria, we noticed that misannotated start codons frequently lead to wrongly assigned SCLs. This and other problems in SCL prediction, such as the relatively high false-positive and false-negative rates of some tools, can be avoided by applying multiple prediction tools to groups of homologous proteins.
Here we present ClubSub-P, an online database that combines existing SCL prediction tools into a consensus pipeline from more than 600 proteomes of fully sequenced microorganisms. On top of the consensus prediction at the level of single sequences, the tool uses clusters of homologous proteins from Gram-negative bacteria and from Archaea to eliminate false-positive and false-negative predictions. ClubSub-P can assign the SCL of proteins from Gram-negative bacteria and Archaea with high precision. The database is searchable, and can easily be expanded using either new bacterial genomes or new prediction tools as they become available. This will further improve the performance of the SCL prediction, as well as the detection of misannotated start codons and other annotation errors. ClubSub-P is available online athttp://toolkit.tuebingen.mpg.de/clubsubp/

Paramasivam N and Linke D (2011) ClubSub-P: cluster-based subcellular localization prediction for Gram-negative bacteria and archaea. *Front. Microbio.* 2:218. doi: 10.3389/fmicb.2011.00218

OTP065

The role of GvpM in gas vesicle formation of Halobacterium salinarum PHH1

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Gas vesicles of Halobacterium salinarum PHH1 are proteinaceous, gas-filled structures containing GvpA and GvpC as the major structural proteins. The hydrophobic GvpA forms the ribbed structure of the gas vesicle wall that is stabilized by GvpC. Twelve additional genes are involved in gas vesicle formation arranged in two clusters gvpACNO and gvpDEFGHIJKLM (= p-vac region). The gvpFGHIJKLM transcript appears earlier compared to gvpDE and gvpACNO mRNAs. GvpM is an essential protein for the gas vesicle formation but produced in small amounts[1]. An alignment of GvpA and GvpM indicates sequence similarities, suggesting that GvpM might be a minor structural component of gas vesicles.
To gain further insights into the role of GvpM, we expressed the p-vac region and an additional gvpM gene inHfx. volcanii transformants. A strong reduction of gas vesicle formation was detected compared to cells expressing p-vac only, but in a few cells two or three extremely long gas vesicles were found. When GvpM was fused to GFP a strong aggregation of GvpM was observed in gvpM-gfp and p-vac+ gvpM-gfp transformants. It is possible that the aggregation of GvpM disturbs the gas vesicle formation. The aggregation of GvpM was also confirmed by Western analysis.
In contrast to the strong reduction of the gas vesicle formation in p-vac +gvpM, transformants expressing p-vac +gvpGHIJKLM contained gas vesicles in normal amounts. These results suggested that additional gene products derived from gvpG-M counteract the aggregation of GvpM. To identify the gene(s) responsible for this effect, transformants containing p-vac +gvpM plus one other gvp gene were analyzed.Transformants harboring p-vac+gvpMG did not produce gas vesicles, whereas the addition of gvpMH, gvpMJ orgvpML led to a wild-type gas vesicle formation. From these results it appears that GvpJ, GvpH and GvpL are able to compensate the inhibitory effect of GvpM on gas vesicle formation in p-vac transformants.
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OTP066

Virus adsorption and elimination in the activated sludge of the municipal wastewater treatment plant of Hannover-Herrenhausen

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The safety of drinking water resources is actually one of the most discussed issues in science. In this context the threat of waterborne disease outbreaks caused by viruses must be particularly considered. The use of bank filtrate for drinking water purpose carries the highest risks of infection because of the clarified, but still viruses containing wastewater in the rivers (1-10 PFU/l at low and up to 10-100 PFU/l at high contaminations) [1]. The most effective approach is to optimize virus reduction during the wastewater treatment process to eliminate the virus load before it is distributed by the water cycle. For this objective we have to determine the elimination and adsorption processes of viruses in the wastewater treatment plant so that we can further on use this knowledge for optimizing the processes within the limits of maintaining the treatment performance.
In the current project we observed in batch experiments with activated sludge the decreasing concentration of somatic coliphages infecting *Escherichia coli* strain WG5 and determined the dependency of adsorption on the total solids content (TS). Furthermore, we also regarded if the virus load is temperature-dependent in the single treatment steps (primary clarifier, activated sludge system, secondary clarifier) of the WWTP Hannover-Herrenhausen (February and August 2011).
The results of the batch experiments demonstrated that after ca. 30d of incubation the elimination process ends, even though not all phages were inactivated (decrease from 1,27 x 10^4 to 2,29 x 10^1 PFU/ml). Consequently, a total virus reduction cannot be achieved within a common sludge age of 12-18d. Concerning the adsorption processes we found that doubling the TS from ca. 3,2 to 6,66 g/l only slightly speeds up the adsorption process. But the finally reached adsorption rates turned out to be equal.
Observing the WWTP indicated, that activated sludge can compensate virus load fluctuations in the primary treatment step over longer periods. In cold season the efficiency of elimination within the WWTP is somewhat lower than in warm season. According to the batch tests the differing TS (February: 3 g/l, August: 4 g/l) have no influence onto the elimination. But the higher temperature in summer leads to increase of bacteria activity, which might be the reason for the better virus elimination.
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OTP067

Hot Metagenomics - towards an archaeal expression host for metagenome analysis

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Archaea offer exciting potential for biotechnological applications, since their proteins, so called “extremozymes”, are active under harsh conditions. Unfortunately, the functional expression of many archaeal (hyper) thermophilic proteins in mesophilic or even thermophilic bacterial hosts is limited. Even more severe is the choice of expression hosts in functional metagenomics. Since Archaea harbor a unique transcription machinery, the use of bacterial expression systems might lead to a pre-selection in current metagenomic approaches. The establishment of an expression platform with a variety of host organisms, including Archaea, will help to capture the natural diversity.
Sulfolobus acidocaldarius is a well characterized thermoacidophilic crenarchaeon that grows optimally at 78°C and pH 2-3. It is genetically tractable and a vector system for protein expression has been established [1].
For the expression in *S. acidocaldarius* the promoter of the maltose binding protein malE is employed. Extensive mutational analysis of different parts of the *malE* promoter including the TATA box, the BRE site and the promoter length resulted in a hybrid promoter that had 5 fold higher expression levels than the wild type promoter. The insertion of the regulator that binds the *malE* promoter, termed MRP (maltose regulatory protein) into the optimized expression vector led to a more than 4 fold increase of expression levels. First results about the expression of archaeal (gluco)amylases, that failed to be expressed in common bacterial and eucaryal expression systems, will be presented. Furthermore, preliminary results about the use of the vector for the expression of metagenomic libraries from hot environments for identifying new and industrially relevant enzymes will be discussed.

OTP068

Microbial biofilm formation in photobioreactors

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Studies regarding the development of biofilms of microalgae and the interaction between prokaryotic and eukaryotic microorganisms are very limited, despite their importance for the development of photobioreactors. In the analyses presented here, the development of biofilm and the bacterial community of the microalgae *Scenedesmus obliquus* and *Chlorella vulgaris* were examined in detail over a time period of three month in a reactor. The diversity and population dynamic of the bacteria were examined through analyses with scanning electron microscope (SEM), fluorescence in-situ hybridization (FISH), denaturating gradient gel electrophoresis (DGGE) and 16S rRNA. Biomolecular analyses indicated that various populations of alpha- and betaproteobacteria (concerning the family of Comamonadaceae) as well as bacteroidetes (e.g. Pedobacter, Sediminibacterium, Flavobacterium and Bacteroidetes that have not been cultivated yet) are associated with the microalgae examined here. However, the populations of alphaproteobacteria (e.g. Sphingomonas, Brevundimonas, Sinorhizobium, Arcicella and Ochrobactrum) as well as the populations of bacteroidetes dominating. Altogether the diversity is rather limited. These results imply that metabolic performance of the bacterial populations is probably related and essential to the growth and stability of the algal culture. In addition, the current work focuses on a detailed metagenome analysis of the algae biofilm communities.

OTP069

The natural transformation machinery in *Thermus thermophilus* HB27: A pilus-independent DNA transporter comprising unique motor ATPase and secretin complexes

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To get insights into the structure and function of DNA translocators we chose the thermophile *T. thermophilus* HB27 as model organism since it exhibits the highest natural transformation frequencies known to date. A genome-wide genetic screen followed by mutant studies led to the identification of 16 distinct competence proteins [1], several of them were found to play a dual role in transformation and piliation. But the question whether the pilus structures itself are essential for DNA uptake was still unanswered.

Here we report on structural and functional analyses of the AAA-ATPase PilF, a unique motor component and the secretin PilQ. Both were found to be essential for natural transformation and piliation. PilF carries a unique N-terminal triplicated GSPII domain and a C-terminal tetracysteinmotif involved in zinc binding [2]. Mutant studies revealed that two of the cysteines are essential for Zn^{2+} binding, piliation, twitching motility and adhesion, but not for natural transformation.
Recently, we reported on the novel structure of a PilQ complex, comprising a stable cone and cup structure and six ring structures [3]. Structural analyses of a set of PilQ deletion derivates in *T. thermophilus* HB27 identified 136 N-terminal residues, encoding an unusual αaβααββα fold as a ring building domain. Deletion of this domain had a dramatic effect on piliation but did not abolish natural transformation.
Taken together, these findings provide clear evidence that the pilus structures are not essential for natural transformation.
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OTP070
Construction of a Bifunctional Cellulase-Xylanase from Thermophilic Microorganisms
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Plant cell walls contain complex polymers and polysaccharides, such as cellulose and hemicellulose. The hydrolysis of these compounds has been shown to be of relevant importance for the industry. Enzymes required for this catalysis are extensively used in different industrial fields ranging from the textile industry to food processing and biofuel production. A number of separate bacterial enzymes work in tandem to efficiently digest polysaccharides, through the hydrolysis of cellulose and hemicellulose. Xylanases hydrolyze β-1,4 glycosidic linkages of hemicellulose, whereas cellulases catalyze random cleavage of the cellulose chain. Few bacteria are able to form multi-component enzyme complexes, known as cellulosomes, while others have separate enzymes or even isozymes working in synergy. Such complexes and processes can be mimicked in laboratories, owing to a number of different molecular and genetic techniques. Several methods, including end-to-end fusion have been shown to generate bi-functional enzyme constructs.
The aim of this study is to generate bi-functional enzyme variants for optimized polysaccharide degradation, by fusing the genes encoding for cellulase and xylanase. A linker, composed of 8 amino acids, is added between the two genes, which can lead to increased stability and flexibility. Here we report the construction of the bi-functional enzymes and their characterization regarding synergestic effects.

OTP071
Ornithine aminotransferase (*rocD*) is essential for optimal growth with arginine as single nitrogen source in *Mycobacterium smegmatis*
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Previously, we studied arginine metabolism under strictly anaerobic conditions, when mycobacteria are unable to replicate but persist instead. However, in the presence of oxygen, mycobacteria show robust growth, even when arginine is present as the only source of nitrogen. The molecular mechanisms for this metabolic activity are unknown. RocD, encoding the ornithine aminotransferase, is part of the arginase pathway, and converts ornithine to glutamate which is subsequently assimilated into central metabolic pathways. A rocD mutant of Bacillus subtilis is not able to utilize arginine as a source of nitrogen.
To investigate the role of ornithine aminotransferase in mycobacteria, a *rocD* mutant in *Mycobacterium smegmatis (Msmeg)* was generated and tested for growth in minimal medium with arginine as a single source of nitrogen. In addition the intra- and extracellular ornithine concentration was measured by gas chromatography mass spectrometry (GC-MS).
The *rocD* mutant of *Msmeg* had a growth defect on arginine, suggesting that *rocD* is essential for arginine assimilation. The mutant also showed an intra- and extracellular accumulation of ornithine, the substrate for the ornithine aminotransferase.
However, we observed residual growth of the mutant on arginine, indicating that in mycobacteria utilization of arginine is more complex than expected. At present we perform experiments to further define arginine metabolism in *Msmeg*.

OTP072
Transport and removal of bacteriophages in saturated sand columns under oxic and anoxic conditions
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To protect groundwater as a drinking water resource against microbiological contamination protection zones are installed. While travelling through these zones concentrations of potential pathogens shall decline to levels that pose no risks to human health. The removal during the subsurface passage is influenced by physicochemical conditions, e.g. oxygen concentration. The survival of microorganisms is affected by the amount of oxygen. In addition, depending on whether dissolved oxygen is present or not, mineral phases with different adsorption properties can be present. In studies examining the transport of virus particles, the RNA bacteriophage MS2 and the DNA bacteriophage ΦX174 are often used because they resemble human viruses in structure and size. Moreover, their detection is much easier and cheaper to accomplish than that of human viruses. Experiments in glass columns (length 55 cm, inner diameter 7.3 cm) filled with medium grained sand were conducted. Different mobile phases either containing dissolved oxygen or being oxygen-free were spiked with bacteriophages MS2 and ΦX174 and pumped through these columns from bottom to top at a filter velocity of about 1m/d. At the effluent physicochemical parameters were measured, and samples for analysing the bacteriophages by plaque assay were taken. Bacteriophage breakthrough curves were compared to breakthrough curves of NaCl, used as a conservative tracer. Both were analysed by one-dimensional models of hydrogeological transport. Total eliminations of bacteriophages were determined by calculating the differences between the input and recovered amounts of viruses. In all experiments, the RNA bacteriophage MS2 was eliminated more efficiently than the DNA bacteriophage ΦX174. Compared to experiments with oxygen-free water, a higher elimination of viruses was observed in oxic water. In connection with batch experiments the data suggest that differences in the inactivation rate coefficients are important to explain the results. Our results will contribute to a better understanding of the transport of viruses through oxic and anoxic zones in the subsurface.

OTP073
Construction of a *Xanthomonas* sp. 35Y rubber oxygenase (RoxA) deletion mutant and improvement of a homologous expression system for RoxA muteins
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Xanthomonas sp. 35Y is the so far only known Gram-negative bacterium capable to degrade natural rubber (polyisoprene) and to use rubber degradation products as the sole source of carbon and energy. The primary attack of the carbon backbone of polyisoprene is catalyzed by a novel type of an extracellular diheme dioxygenase (rubber oxygenase RoxA) [1-3]. To investigate the unknown RoxA cleavage mechanism, structure-function analysis of RoxA muteins is necessary. Unfortunately, heterologous expression of RoxA was not possible, neither in *Escherichia coli*, *Bacillus subtilis* nor in *Pseudomonas putida*. Therefore, a homologous RoxA expression system was established in the host strain *Xanthomonas* sp. 35Y [4]. However, it turned out that expression of a *roxA* copy from a broad host range plasmid (with rhamnose-dependent promoter) transferred to the *Xanthomonas* strain could not be obtained immediately. Only after spontaneous integration of the plasmid (after weeks up to months and several transfers on solid media) into the chromosome, stable rhamnose-dependent RoxA expression was obtained. Thus, a *roxA* deletion mutant was constructed using sucrose counter selection with *sacB*. To improve the efficiency of integration of the expression plasmid into the chromosome, the phage PhiC31 integration system was applied. Using this system, we succeeded in rapid and reproducible integration of *roxA* copies into the *Xanthomonas* sp. chromosome. Wild type RoxA and first RoxA muteins were successfully expressed. High yields of recombinant wild type RoxA (≥ 1 mg/L culture) were reproducibly obtained within 2-3 days of cultivation in the presence of rhamnose. Purified recombinant RoxA was active, its activity could not be distinguished from RoxA that had been purified from *Xanthomonas* sp. wild type.
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OTP074

Comparison of Faecal Culture and Real-Time Quantitative PCR Methods for Detection of *Mycobacterium avium* subsp. *paratuberculosis* in Bovine Faecal Samples

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Mycobacterium avium subsp. *paratuberculosis* (MAP) is a robust microorganism, which causes incurable chronic enteritis in cattle. The present study compared the efficacy of two different faecal culture procedures and Taq-Man PCR assay (Applied Biosystem) for detection of MAP in faecal samples. Sixty one faecal samples were collected from two Dutch cattle herds (n=40, and n=21, respectively) which are known to be MAP positive. For culturing, all individual samples were decontaminated using 0.75% HPC and cultured on HEYM agar (Harold's Egg Yolk Medium containing Mycobactin J and AVN, Becton Dickinson). The second cultural method in sequentially two decontamination steps used 4% NaOH and malachite green-oxalic acid cultured on HEYM agar and on LJ agar (modified Löwenstein-Jensen media contain Mycobactin J). For the Taq-Man real-time PCR method, all faecal samples were tested in two different laboratories using the same PCR kit. The sensitivity of the two cultural methods were 1.6% (n=1/61), 4.9% (n=3/61) and 8.2% (5/61) of HEYM/ 0.75% HPC; HEYM/ 4% NaOH/malachite green-oxalic acid and LJ/ 4% NaOH/malachite green-oxalic acid, respectively. The sensitivity of the Taq-Man real-time PCR in two different laboratories were 13.1% (n=8/61) and 16.4% (n=10/61). The results revealed that cultural method using LJ/ 4% NaOH/malachite green-oxalic acid is more sensitive than others and the Taq Man PCR assay had higher specificity than the cultural methods. The results showed a significant deference between Taq-Man real-time PCR assay and two cultural methods. In conclusion, Taq-Man real-time PCR on bovine faecal samples is a fast reliable method and could be applied in routine screening of MAP, leading to the improvement of the efficiency of MAP control strategies.

OTP075

Multilocus Sequence Typing (MLST) for the infra-generic taxonomic classification of entomopathogenic *Rickettsiella* bacteria

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The taxonomic genus *Rickettsiella* comprises intracellular bacteria associated with a wide range of arthropods that are currently classified in four recognized species - namely the nomenclatural type species, *Rickettsiella popilliae* (Dutky & Gooden), as well as *Rickettsiella grylli* (Vago & Martoja), *Rickettsiella chironomi* (Weiser), and *Rickettsiella stethorae* (Hall & Badgley) - and numerous further pathotypes. Both the delineation of species and the synonymization of pathotypes with species are highly problematic.
In the sequel of a previous phylogenomic study at the supra-generic level, nine selected genes - the 16S and 23S rRNA genes and the protein-encoding genes *dnaG, ftsY, gidA, ksgA, rpoB, rpsA*, and *sucB* - were evaluated for their potential as markers for the generic and infra-generic taxonomic classification of *Rickettsiella*-like bacteria. A methodological approach combining phylogenetic reconstruction with likelihood-based significance testing was employed on the basis of sequence data from the *Rickettsiella popilliae* - synonymized pathotypes `*Rickettsiella melolonthae'* and `*Rickettsiella tipulae´* as well as the species *R. grylli*.
The study identified two genetic markers, *gidA* and *sucB*, for MLST analysis within the bacterial genus *Rickettsiella*. In contrast, *rpsA* and *ftsY* gene sequences were found to be sufficiently phylogeny-informative to produce a significant genus-level classification of *Rickettsiella*-like bacteria. Both the *gidA* and *sucB* genes were shown to be highly phylogeny informative at the infra-generic taxonomic level and have been subject to functional selection as concluded from their non-synonymous : synonymous site substitution frequencies (d_N/d_S) of 0.21 and 0.31, respectively. Moreover, being located at a distance of 570 kbp from each other in the *R. grylli* genome (app. 1.5 Mbp), the simultaneous use of both markers will make it likely that possible LGT events will not have affected both genes at a time. In particular, on the basis of the above analysis and within the range of infra-generic diversity covered by the present study, these markers' reliability and resolution potential for taxonomic studies within the genus *Rickettsiella* appear higher than those of the corresponding 16S rRNA-encoding sequences.
Reference: Leclerque A, Hartelt K, Schuster C, Jung K, Kleespies RG (2011) Multilocus sequence typing (MLST) for the infra-generic taxonomic classification of entomopathogenic *Rickettsiella* bacteria. FEMS Microbiology Letters 324:125-134.

OTP076

Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) for Species Identification of Bacteria of Genera *Arcanobacterium* and *Trueperella*

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Genus *Arcanobacterium* (*A.*) consisted of nine species, it was split in two distinct phylogenetic lineages *Arcanobacterium* and *Trueperella* (*T.*) in 2011. Species *A. phocae*, *A. pluranimalium*, *A. hippocoleae*, *T. pyogenes*, *T. bonasi*, *T. bialowiezensis* and *T. abortisuis* were mainly recovered from infections of various animals and *A. haemolyticum* and *T. bernardiae* generally cause diseases in humans. In the present study Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) was evaluated to identify 121 isolates and 11 reference strains of genus *Arcanobacterium* and genus *Trueperella*. All 121 isolates were recovered from different animals and previously classified phenotypically and genotypically to six species of both genera. Species identification by MALDI-TOF MS was carried out by comparing the main spectrum of each isolate with the main spectra of 11 *Arcanobacterium* or *Trueperella* reference strains obtained in the present study and 3740 database entries included in the MALDI Biotyper 2.0 software package (version 3.1.1.0) (Bruker Daltonik GmbH, Bremen, Germany). MALDI-TOF MS correctly identified (log (score) values ≥ 2.0) 22 of 23 *T. abortisuis* isolates and all investigated isolates of the species *A. haemolyticum* (n= 10), *A. pluranimalium* (n = 1), *T. bialowiezensis* (n = 3), *T. bonasi* (n = 7), and *T. pyogenes* (n = 77). According to the present results MALDI-TOF MS is a promising tool for fast and reliable identification of species of *Arcanobacterium* and *Trueperella*. Further studies with additional isolates, also including *Arcanobacterium* and *Trueperella* species commonly related with human infections, would underline the robustness of MALDI-TOF MS for identification of bacteria of both genera.

OTP077

Will not be presented!

OTP078

Identification of *Campylobacter* Species from Zoo Animals by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry

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The identification of genus *Campylobacter* at species level in routine diagnostic laboratories using conventional methods is still problematic due to their poor biochemical activity. In this study, a total of 32 faecal samples from 32 wild animals were examined during routine microbiological diagnosis. Five isolates were suspected *Campylobacter* strains isolated from five animals (monkey, trumpeter swan, leopard and two meerkats). For species identification, Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) and DNA sequencing techniques were used. Two *Campylobacter upsaliensis* affiliated and two *Campylobacter jejuni* strains were identified. MALDI Biotyper software resulted in no reliable identification for isolate OV50-1. Indeed, this isolate may represent a new species of the genus *Campylobacter*. Partial 16S rRNA gene sequence similarity was only 97.7% to *C. upsaliensis*, the best match of GenBank database comparison. This underlines that there are no false-positive identification results by MALDI Biotyper software. According to the present results MALDI-TOF MS is a fast and reliable method for identification of bacteria of genus *Campylobacter* at the species level in routine diagnostic laboratories and might help to elucidate the role of *Campylobacter* in infections even of exotic species.

OTP079

Oil degradation by *Alcanivorax borkumensis* - Understanding stress response networks in relation to catabolic performance

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The recent Gulf of Mexico oil spill about two years ago has once again shown the urgent need for simple and efficient bioremediation techniques that can be quickly implemented on a large scale. The unprecedented continuous flow of crude oil into the Gulf of Mexico presented a huge challenge to existing oil-spill treatment methods, and current technologies were not able to cope with the size and nature of the oil spill. Although general interest about polluted environments has lessened over the past decade, it is nonetheless necessary to make controlled interventions and avoid pollution damage.
A distinct group of members of the *Oceanospirillales* have a high affinity towards oil hydrocarbon substrates in seawater. The dominating species in this community is *Alcanivorax borkumensis* (Yakimov *et al*., 1998), which has been studied intensely for its bioremediation potential (Gertler *et al*., 2009, 2010). Unfortunately various stress conditions that naturally can be found in seawater, were so far not taken into account for bioremediation studies. The aim of our project is to understand stress and survival to finally improve catabolic performance in the field and find optimal formulations.
For *Alcanivorax borkumensis* a microarray design was constructed to gain insight into stress networks under environmental conditions. First stress-response networks and the post-translational regulation of stress response mechanisms were revealed.
Also membrane fatty acid composition was investigated and a mechanism to cope harsh stress conditions was found. Finally first attempts for a robust formulation of *Alcanivorax borkumensis* were started with the aim to use those formulations in field trials for oil spills.

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Yakimov MM, Golyshin PN, Lang S, Moore ER, Abraham WR, Lunsdorf H & Timmis KN (1998) *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant producing marine bacterium. Int J Syst Bacteriol 48: 339-348.

OTP080

Development of an in situ remediation technology for BTEX-contaminated groundwater by the use of iron oxide nanoparticles

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Iron oxides play an important role in the global biogeochemical cycles. In recent years, a lot of iron-reducing bacterial strains were discovered and it became obvious that dissimilatory Fe(III) reduction plays a significant role in anaerobic respiration processes in anoxic subsurface environments. The reduction of Fe(III) is coupled to the oxidation of natural organic matter or organic pollutants, whereby carbon dioxide is produced.However, Fe(III) in natural iron oxide minerals is poorly soluble, shows a high crystallinity and is thus hardly bioavailable for microorganisms.
A recent study showed that colloidal iron oxide nanoparticles exhibit an exceptionally high reactivity compared to the reactivity of macro-sized ferric iron present in bulk phases (Bosch *et al*., 2010).
Here, we want to use this high reactivity of iron oxide colloids for a new remediation technology for BTEX-polluted groundwater horizons. Eventually, our aim is to produce highly reactive iron oxide colloids which can be injected into a contaminant plume in order to stimulate the microbial iron reduction and degradation of contaminants.
In initial growth batch experiments cells of *Geobacter metallireducens* or *Geobacter toluenoxydans* were concentrated to high density, repeatedly washed and added to toluene containing reaction medium with Fe(III) either in the macromolecular state or in the nanosized form. Iron reduction was constantly measured over a period of around 1000 h, using a ferrozine assay for Fe(II) formation. Besides, toluene-degradation was analysed by GC-MS measurements and carbon isotope fractionation. These experiments demonstrated that the addition of nanosized ferrihydrite enhanced the microbial toluene degradation, compared to the corresponding bulk macroaggregates. Toluene was depleted almost completely by the use of nanosized ferrihydrite, whereas bulk ferrihydrite showed no significant degradation.
In the next step the iron oxide nanoparticles will be exposed in 2D-aquifers to examine their long-term stability as well as their long-term reactivity. Because of natural sediment as a matrix for the 2D-aquifer also a catalytic effect is possible. As a last step we plan an outdoor test at a BTEX-contaminated site of a former industrial area.

Bosch *et al*., 2010; Appl. Environ. Microbiol. 76:184-189.

OTP081

A new clean deletion and expression system for different *Gluconobacter oxydans* strains

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The acetic acid bacterium *Gluconobacter oxydans* is well known for its ability to incompletely oxidize a great variety of carbohydrates, alcohols and related compounds. In a multitude of biotechnological processes *G. oxydans* is used because of its regio- and stereo-selective oxidative potential. The incomplete oxidation of substrates is catalyzed by various membrane-bound dehydrogenases.
For the detailed investigation of *Gluconobacter* a well established and easily applicable clean deletion system is essential.
A method for markerless clean deletion in *G. oxydans* strain 621H is already available from our group. This method is based on the use of uracilphosphoribosyl transferase (UPRTase) as a counter-selectable marker in the presence of the toxic pyrimidin analogue 5-fluorouracil (5-FU). The method is restricted to the usage of previously generated mutants of the UPRTase gene (Δupp).
To allow usage of wild-type strains instead of Δupp-mutants, we developed an improved clean-deletion system using a cytosine-deaminase as the counter-selectable marker in the presence of toxic 5-fluorocytosine (5-FC).
In order to complement deletions of membrane bound dehydrogenases we constructed a shuttle vector system for their functional expression. This system was successfully used for the complementation of membrane bound dehydrogenases in *G. oxydans* 621H and could also be used in other *G. oxydans* strains. Furthermore this vector system is available for the expression and characterization of membrane bound dehydrogenases from a vinegar metagenome

OTP082

Will not be presented!

OTP083

Roast Duck with Curry Aromatized on Grapefruit Gravyor: How to Properly Keep Research Records

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Any experimental work performed in a laboratory needs to be properly documented. Not only to assist a researcher in recalling each individual step of an experiment after weeks, months, or even years, but also because someday its results might become part of a publication. Therefore, keeping a laboratory notebook is a fundamental aspect of good scientific praxis, and a clearly written, self-explanatory lab book should be the standard. However, from our everyday experience with students, both new and advanced in the lab, we have learned that a clear lab book does not come naturally: dates are missing, objectives of experiments are not indicated, methods are not described in detail, reasons for certain procedures remain unclear, samples have been arbitrarily renamed, supplementary documents necessary for data interpretation are missing, loose notes, printouts, and photocopies fall out when flicking through the lab book, interim results are not documented, the writing is illegible, and so on. To alleviate this situation and help our students to avoid the most common flaws, we have formulated a few general guidelines on how to properly keep research records that we think are particularly crucial. So far so good! However, modern psychology posits that guidelines are not well adopted as long they are perceived as just another “dull instruction”. Besides, students are potentially overloaded with “stuff to read and keep in mind”. Therefore, we were especially concerned with the task on how to get our points across without creating a feeling of something that “must” be followed or “should” be done, without being boring, and without being wiped out of memory shortly after reading. We encountered this challenge by (i) visually exemplifying our points in the form of a poster, (ii) wrapping our guidelines into a humorous story of metaphoric character, and (iii) placing the poster in the lab floor, thereby reminding our students of the most important points on a daily basis. Our poster entitled “Roast Duck with Curry Aromatized on Grapefruit Gravy” features a young scientist who keeps a cook book to keep record of her attempts to fix a decent dinner for friends at her place, eventually doing everything right, except for … To find out come and see our poster!

OTP084

The Use of GFP-GvpE fusions to characterize the interaction of the two regulatory proteins GvpD and GvpE of *Halobacterium salinarum*

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Gas vesicle formation in *Halobacterium salinarum* involves fourteen *gvp* genes arranged in two oppositely oriented gene clusters *gvpACNO* and *gvpDEFGHIJKLM*. The expression of these genes is regulated by two endogenous regulatory proteins, GvpD which is involved in repression and the transcriptional activator GvpE. Both proteins are able to interact and this interaction results in the lack of GvpE in *Haloferax volcanii* D + E transformants [1, 2]. To quantify the reduction of the amount of GvpE in the presence of GvpD, an N-terminal fusion of GFP to GvpE was constructed. The activating function of the GFP-E fusion protein was shown in P_A-*bgaH* transformants using the β-galactosidase activity of BgaH as reporter, since wild-type GvpE (E_{WT}) or the GFP fusion GFP-E resulted in similar BgaH activities. The amount of GFP-E was then quantified by fluorescence measurements in the absence or presence of $GvpD_{WT}$ or GvpD mutants. GvpD mutant $D_{3\text{-}AAA}$ acts as a superrepressor, whereas two other GvpD mutants, D_{Mut1} and D_{Mut6}, lack the repressing function. The fluorescence level determined for the GFP-E transformant was set 100%, the fluorescence of GFP-E + D_{WT} was reduced to 40% in transformants, whereas the superrepressor $D_{3\text{-}AAA}$ reduced the fluorescence of GFP-E to 20%. No reduction of fluorescence was observed in GFP-E transformants carrying the defective mutant proteins D_{Mut1} or D_{Mut6}. The GFP-E reporter system was also used to study the effect of various GvpE mutants that lost their activating function. The GvpE mutants E_{ARA} and E_{K104A} incurred mutations in one of the two putative DNA binding regions and is completely abolishing the activator function. D_{WT} and also the superrepressor mutant $D_{3\text{-}AAA}$ reduced the fluorescence of both GvpE mutants to 50% and 40%, respectively. Further studies on GvpE and GvpD mutants are under way, to search and identify contact sites in both regulatory proteins that are important for the reduction of GvpE.

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OTP085

Enhanced FMN-binding fluorescent proteins

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Fluorescent proteins (FP) like the green fluorescent protein (GFP) and its variants are widely used *in vivo* reporters to study protein expression, localization and interaction [1]. Flavin-mononucleotide (FMN)-binding FPs (FbFPs) are a new class of fluorescent reporters, which are derived from bacterial 'Light Oxygen Voltage' (LOV) photoreceptor domains. In contrast to the well-established FPs of the GFP-family, FbFPs do not require molecular oxygen for the development of their fluorescence signal and are therefore suitable reporter proteins for fluorescence imaging under aerobic, as well as under anaerobic conditions [2]. Applicability of FbFPs has so far been demonstrated for various anaerobic bacteria [2-4] and yeast [5]. Their independence of molecular oxygen also enables them to outperform GFP-like proteins as quantitativein vivoreal-time reporters [6]. We are now conducting directed evolution experiments, in order to further enhance the fluorescence properties of FbFPs. Here, we report on the development of novel enhanced FbFP derivatives exhibiting improved brightness or blue-shifted absorption and fluorescence spectra.

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5. Tielker D, Eichhof I, Jaeger KE, Ernst JF:Flavin mononucleotide-based fluorescent protein as an oxygen-independent reporter in Candida albicans and Saccharomyces cerevisiae.Eukaryot Cell2009,8(6):913-915.
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OTP086

Interaction of engineered anorganic nanoparticles with bacterial biofilms

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In the last decade engineered inorganic nanoparticles (EINP) have been brought to the market in large quantities partly used as agents with antibacterial properties (e.g. Ag-EINP). Today, the resulting ambient concentration of Ag nanoparticles in river water is estimated in a quite broad range from 0.01 µg/l up to 300 µg/l [1, 2]. Based on a potential pollution rate of EINP, bacterial biofilms in the environment will certainly be encountered as well. These Biofilm communities ensure essential ecosystem functions of lakes and rivers (e.g. self purification) and provide important ecosystem related services for drinking water reservoirs, recreational centres, and biodiversity resources [3]. The biofilms display the locality where adsorption of organic and inorganic matter does occur, and microbially mediated degradation, metabolism and mineralisation take place. Even though numerous studies have been dealt with biological effects of Ag nanoparticles already, knowledge of the ecotoxicology of EINP to bacteria is mostly limited to studies on single bacterial species in liquid cultures [4].
To investigate interactions of these widely used EINP at the aquatic-terrestrial interface, the DFG research unit INTERNANO was approved very recently. The study presented here is part of the preliminary work of the respective subproject BIOFILMS. To get first insights into the mutual effects of EINP on structure and function of biofilms, both monospecies biofilms and native biofilms obtained from the river Rhine were investigated after exposure to different concentrations of Ag nanoparticles. *Aquabacterium citratiphilum* was used as model organism to generate biofilms under laboratory conditions applying commercial drip flow biofilm reactors (BioSurface Technologies, Inc.). By using fluorescent dyes SYBR® Green and epifluorescence digital imaging we obtained information regarding the amount of adhered biomass. Application of LIVE/DEAD®BacLight™ Bacterial Viability Kit showed the relative amount of bacteria with uncompromised membrane integrity and therefore gave an estimate of the active total biofilm volume to surface area. From these results, effective concentration of Ag nanoparticles on the adhered microbial biomass can be estimated for the model system investigated.

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OTP087

Construction of an expression system based on mannitol PTS in *Bacillus subtilis* and its regulation

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Regulation of the mannitol utilization system (*mtl* operon) in *B. subtilis* was studied in order to construct an expression system. The *mtl* operon consists of *mtlA* (encoding the phosphoenolpyruvate-dependent phosphotransferase system (PTS) enzyme $IICB^{Mtl}$), *mtlF* (encoding the PTS enzyme IIA^{Mtl}), and *mtlD* (encoding the mannitol 1-phosphate dehydrogenase). The *mtlAFD* operon is activated by MtlR (encoding by *mtlR*), which is a transcriptional activator containing so-called PTS regulatory domains (PRDs) as well as $EIIB^{Gat}$ and $EIIA^{Mtl}$ domains. Principally, the phosphorylation state of the domains of such activators regulates its function. In other words, phosphorylation of PRDII activates a PRD containing activator, while phosphorylation of other domains deactivates it. In this study, the promoters of *mtlAFD* operon (P_{mtlA}) and *mtlR* (P_{mtlR}) were fused to *lacZ* as a reporter gene. Measurement of β-galactosidase indicated that the P_{mtlA} and P_{mtlR} were induced by mannitol, whereas glucose repressed their activities. Using primer extension method, transcription start sites as well as -10 and -35 boxes were identified indicating a σ^A-like structure of P_{mtlA} and P_{mtlR}. Specific regulation of P_{mtlA} and P_{mtlR} were investigated by deletion of *mtlAF*, *mtlD* and *mtlR* or mutation of mtlR to *mtlR*-H342D (as a phosphorylated PRDII mimic). Here, it was observed that the deletion of $EIICB^{Mtl}$ and $EIIA^{Mtl}$ components and MtlR-H342D mutation resulted in constitutive expression of P_{mtlA} and P_{mtlR}, while deletion of *mtlR* strongly reduced the promoter activity. Subsequently, the effect of carbon catabolite repression (CCR) was investigated wherein generaltrans andcis components of CcpA-dependent CCR, and *ptsG* (encoding glucose transporter)were deleted or mutated. Altogether, the results indicated that glucose repression was mainly caused by an inhibition of MtlR by PtsG, while CcpA-dependent CCR components exhibited minor effects. Consequently, we assume that the phosphorylation state of PRDII domain (H342) plays the main role in glucose repression of mannitol system.

OTP088

Self-inducible *Bacillus subtilis* expression system

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High product yields and low costs are two of the main objectives of an economic production process. Hence, we developed a novel technically

compliant expression system for heterologous protein production in *Bacillus subtilis* [1].The system involves the positively regulated *manP* promoter of the mannose operon of *B. subtilis*. The enhanced green fluorescent protein (eGFP) was chosen as a reporter by reason of an easy online-tracking of its expression. With the wildtype strain relatively high product yields of 5.3 % [5.3 g_{eGFP} per 100 g_{cdw} (cell dry weight)] were achieved but required large quantities of mannose to induce the reactions, thus rendering the system's technical application rather expensive. Trying to improve this, mutant *B. subtilis* strains were used: the Δ*manA* (mannose metabolism) strain TQ281 and the Δ*manP* (mannose uptake) strain TQ356. The total amount of inducer was reduced with TQ281. However, the strain displayed sensitivity to the inducer mannose. To further improve the cost-efficiency and product yield of the system, an inducer-independent self-induction system with TQ356 was developed, in which glucose prevents induction by carbon catabolite repression. Optimal self-induction conditions could be achieved by utilizing a glucose limited process strategy, namely a fed-batch process. The self-induction was initiated at the beginning of the glucose-restricted transition phase between the batch and fed-batch phase of fermentation and was maintained throughout the entire glucose-limiting fed-batch phase. With this strategy a nearly threefold increase of product yield to 14.6 % was gained.
The novel *B. subtilis* self-induction system thus makes a considerable contribution to improve product yield and to reduce the costs associated with its technical application.
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OTP089

Biological roles of sRNAs in the halophilic archaeon *Haloferax volcanii* and identification of potential mRNA targets

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Bioinformatic approaches and/or experimental studies led to the identification of many small non-coding RNAs (sRNAs) in several archaeal species. The halophilic archaeon *Haloferax volcanii* contains a high number of about 350 sRNAs, indicating the importance for RNA-mediated regulation in these species [1, 2].
To unravel biological roles of haloarchaeal sRNAs more than 30 sRNA gene deletion mutants were constructed and their phenotypes were compared to that of the wild-type under 14 different conditions. For example, growth on different carbon sources, growth at different salt concentrations, and stress adaptation were characterized. This phenotyping approach was enabled by the possibility to grow *H. volcanii* in microtiter plates, which allows highly parallel cultivation [3]. Length of the lag phases, growth rates, and growth yields were quantified. In addition, cell morphology was analyzed microscopically and cell behaviour was characterized using swarm plates. For 25 deletion mutants phenotypic differences to the wild-type were discovered, which in all cases were specific for one or a few of the tested conditions. Notably, in several cases deletion of a sRNA gene resulted in a gain-of-function phenotype, which has not been described for any bacterial sRNA gene deletion mutants. The mutant phenotypes revealed that sRNAs are involved in many biological processes in haloarchaea, including stress adaptation, metabolic regulation and cell behaviour. Selected examples will be shown.
In archaea no target molecules for sRNAs have been identified so far. The search for potential mRNA targets of *H. volcanii* sRNAs included the analysis of sRNA gene deletion as well as overexpression mutants using microarrays and Northern blot analyses. In addition, bioinformatic approaches were applied. The combination of the different methods led to the identification of putative targets, and future research will aim at characterization of the predicted sRNA-mRNA interactions.

OTP090

Comparison of two *Dehalococcoides* isolates from the Bitterfeld region of Germany: reductive dehalogenase genes encoded in the genomes and the capacity to dechlorinate dibenzo-*p*-dioxins

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The quality of both groundwater and surface water is severely jeopardized globally through contamination by halogenated compounds. The industrial area of Bitterfeld in central Germany is particularly highly contaminated by different chlorinated benzenes and ethenes and also by very persistent compounds like polychlorinated dibenzo-*p*-dioxins and dibenzofurans. The genus *Dehalococcoides* represents a novel group of strictly anaerobic bacteria, which are key naturally occurring bioremediators of these highly toxic compounds. Recently, two *Dehalococcoides* strains, DCMB5 and BTF08, have been enriched and isolated from samples taken from sediment and aquifer, respectively, at the contaminated site. Strain DCMB5 (1) is able to dechlorinate hexa- and pentachlorobenzene, as well as all three tetra- and one trichlorobenzene. In contrast, strain BTF08 is adapted to the dechlorination of chlorinated ethenes (2).
Comparison of the genomes of both strains revealed a contextually conserved core with some marked differences such as the presence of three copies of a transposable element in strain BTF08 and of a CRISPR locus in strain DCMB5. The genes encoding homologues of reductive dehalogenases (Rdh) are mostly located in so-called high plasticity regions (3). Only eight of the 20 Rdhs in BTF08 have orthologues in strain DCMB5 according to the orthologue definition of Kube *et al*. (4). One Rdh of strain BTF08 has no orthologue in other *Dehalococcoides* strains. The occurrence of orthologues of the functionally characterized CbrA, a chlorobenzene Rdh, only in strain DCMB5 and the combination of both TceA and PceA (tri- and tetrachloroethene Rdhs, respectively) only in strain BTF08 is in agreement with the different physiologies of both strains. Strain DCMB5 was shown to dechlorinate selected dibenzo-*p*-dioxins. To link further differences in the content of *rdh*-genes of both strains to their dehalogenation capacity we compared the capability of both strains to dechlorinate different chlorinated dioxins.
(1) Bunge *et al*. (2008) Environ Microbiol 10, 2670-2683
(2) Cichocka *et al*.(2010) FEMS Microbiol Ecol 72, 297-310
(3) McMurdie *et al*. (2009) PloS Genet 5, e1000714
(4) Kube *et al*. (2005) Nat Biotechnol 23, 1269-1273

OTP091

Archaeal versus bacterial ammonia oxidation in oligotrophic and eutrophic freshwater sediments

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Ammonia oxidation, the first and rate-limiting step of nitrification, is carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). Recent studies from marine and terrestrial environments suggest that AOA are better adapted to conditions of low ammonia availability. However, only little is known about the respective role of AOA versus AOB in freshwater environments. We carried out comparative investigations of the abundance and activity of AOA and AOB in sediments of a eutrophic and a neighbouring oligotrophic lake located in Northwest Germany, addressing two hypotheses: (i) AOA/AOB ratios shift in favour of AOB with increasing ammonium availability, and (ii) AOA play a major role in ammonia oxidation in the oligotrophic lake while AOB dominate this process in the eutrophic lake. Abundance, transcriptional activity, and community composition of AOA and AOB were analyzed targeting the *amoA* gene encoding ammonia-monooxygenase as a functional marker. AOB-*amoA*/AOA-*amoA* gene ratios ranged from 1 to 1000 in the sediment of the eutrophic lake and from 0.001 to 1 in the oligotrophic lake. Here, AOA were especially abundant in rhizosphere sediment where they constituted up to 50 % of the total archaeal population. AOB-*amoA* gene copy numbers as well as AOB-*amoA*/AOA-*amoA* gene ratios were positively correlated to NH_4^+ concentrations in the sediment pore water across sites. Sediment samples taken directly from the field site or from short-term incubation experiments showed higher transcriptional activity of AOA or AOB in the oligotrophic and eutrophic sediments, respectively. These findings are supported by preliminary results from potential nitrification assays using specific inhibitors of bacterial ammonia oxidation, which suggest that AOB dominate ammonia oxidation under eutrophic conditions.

OTP092

Bacterial formation of biogenic amines in grape juice: the influence of culture conditions

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The production of biogenic amines by lactic acid bacteria was analyzed under various culture conditions. For the derivatization of the biogenic amines, a freshly prepared solution of solid ortho-phthaldialdehyde was used. This led to more constant results compared to the use of commercially available reagents. After microbial growth the produced biogenic amines were purified by solid phase extraction (SPE). A novel method including the application of a strong cation exchange cartridge was developed which was less time-consuming than earlier described methods. The samples were analyzed with reversed phase high performance liquid chromatography (RP-HPLC). The investigations showed that the content of biogenic amines depended on the growth temperature, the pH value and the amino acid content. At 25 ºC higher concentrations up to factor of 16 of biogenic amines were produced by the selected microorganisms

compared to 20 ºC. An increase of the pH-value from 3.5 to 3.7 led to higher formation of biogenic amines of at most 100%. In addition, the tested strains produced a maximum of 170 mg/l of the health-relevant tyramine, when amino acids including tyrosine were added.

OTP093

Impacts of climate-sensitive environmental factors on fecal bacterial loads in the river Lahn

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Fecal pollution of rivers bears a public health risk and is diminishing the quality of ecological services provided by these important ecosystems [1]. Therefore the aim of this study is the evaluation of potential impacts of climate change on the microbial water quality of rivers on an experimental basis.
To get a deeper understanding of the hygienic state of a subset river and how it is influenced by climatic relevant factors, a 12 months-monitoring is ongoing.
During this monitoring, water samples are taken weekly at five different sites of the river Lahn.
Relative abundances of fecal indicator organisms comprising *E. coli*, somatic coliphages and intestinal Enterococci are determined. Presence of enterohaemorrhagic *E. coli* (EHEC) is tracked by means of a commercial PCR assay. In addition, the amount of settleable particles and physical-chemical parameters such as temperature and pH are measured.
Main focus of the analysis is to investigate the influence of climate sensitive factors such as water temperature, rainfall and water discharge on the relative abundance and spatial distribution of fecal indicator bacteria.
To assess the public health risk and for the development of managing strategies it is important to identify sources of fecal pollution as well. Microbial source tracking by means of qPCR will be performed on the basis of signature sequences specific for *Bacteroidetes* in order to discover contamination pathways.
Preliminary data show that the amount of particles and the relative abundance of indicator organisms increase with river discharge. Accordingly, highest rates could be observed after rainfall events. On the other hand, declining water temperatures also seem to have a positive impact on coliphages.
Ongoing mesocosm experiments will aim at answering the question whether the high counts after rainfalls can be traced back only to the input from external sources or if they are at least partly caused by resuspension from the sediment.
In general, numbers of *E. coli* were higher than those of coliphages and Enterococci. In particular cases they exceed the EU directive for bathing waters.
In addition, results obtained with a commercial PCR assay indicate the presence of *E. coli* O104.

[1] Gerbersdorf S. U., Hollert H., Brinkmann M., Wieprecht S., Schüttrumpf H., and Manz W. (2011). Anthropogenic pollutants affect ecosystem services of freshwater sediments: the need for a "triad plus x" approach. *Journal Soils Sediments* doi 10.1007/s11368-011-0373-0.

OTP094

Active groundwater bacterial communities in karstic aquifers in the Hainich (Thuringia, Germany)

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Groundwater ecosystems harbour a great proportion of the Earth`s prokaryotic biomass, however, knowledge about microbial diversity in pristine groundwater is scarce. In these ecosystems, characterized by low organic carbon content, lithoautotrophy might represent an important microbial metabolic strategy. This study aimed to investigate the diversity of active groundwater bacteria by targeting gene transcripts for 16S rRNA and RuBisCO II large-subunit (cbbM) responsible for CO_2-fixation in the Calvin Cycle. Groundwater was collected in November 2010 and April 2011 from different wells along a land-use gradient, ranging from forest to agricultural fields, following groundwater flow at the northeastern slope of the Hainich region (Thuringia). The groundwater had a pH between 7.2 and 7.4, contained low organic carbon, had 30% oxygen saturation, and contained up to 30 mg/l dissolved CO_2. Analysis of the active community members by pyrosequencing revealed great spatial variability in the diversity of active groundwater bacteria as well as differences between the autumn and spring samples. In contrast, the composition of bacterial communities expressing the RuBisCO II gene showed only little variation between sampling sites and time points. More than half of the 16S rRNA sequences (51.4%) were affiliated with Proteobacteria (dominated by the β- and δ-subgroups) and unclassified Bacteria were also detected in high numbers (23.6%). RuBisCO II sequences were related to the generaThiobacillus andSulfuricella and tocbbM sequences of uncultured bacteria originating from hypersaline water and tar oil-contaminated aquifers. We are currently working with multivariate statistics to correlate microbial observations with long-term physiochemical data sets to reveal the possible impact of land use on the groundwater bacterial communities.

OTP095

Will not be presented!

OTP096

Efficient marker recycling using the optimized FLP/*FRT* recombination system in filamentous fungi

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The genetic manipulation of many filamentous fungi is limited by the number of functional resistance marker. For this reason we have developed a marker recycling for the penicillin producer *Penicillium chrysogenum* based on the FLP/*FRT* recombination system from the yeast *Saccharomyces cerevisiae*. In a first approach the functionality of the system was tested. Therefore a nourseothricin resistance cassette flanked by *FRT* sequences in direct repeat orientation (*FRTnat1* cassette) was ectopically integrated into a *P. chrysogenum* recipient strain. The corresponding transformants were used to complete the system by transforming a codon-optimized *Pcflp* recombinase gene. Our analysis of several transformants showed that successful recombination events were achievable with the codon-optimized recombinase. To further extend the application of the FLP/*FRT* recombination system, we generated a marker-free ΔPcku70FRT2 strain which enables the production of multiple deletion strains by highly efficient homologous recombination. Moreover we have established a one-step marker recycling. For this purpose the FLP/*FRT* system and the *nat1* marker gene were combined in a so-called *nat1*-Flipper cassette. To regulate the recombinase gene expression the inducible *xyl* promoter was used. In future we intend to use different Flipper cassettes together with the ΔPcku70FRT2 strain to construct marker-free double and triple mutants.
Furthermore the applicability of the developed marker recycling system was demonstrated in the ascomycetes *Sordaria macrospora* and *Acremonium chrysogenum* indicating, that the optimized FLP/*FRT* recombination system will be suitable to fungi unrelated to the species investigated in this study.

OTP097

Methanogenic archaea from Siberian permafrost: unveiling biosignatures using Raman spectroscopy

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The Mars Express and Phoenix Missions have reported the presence of permafrost sediments as well as high levels of methane in certain areas of the northern hemisphere of the Red Planet. The fact that methane breaks up with UV-light and has a chemical lifetime of about 300 to 600 years in the Martian atmosphere is of great interest because of its potential biological origin (although geochemical explanations may also be possible but have not been confirmed yet).
Methanogenic archaea from Siberian permafrost have been recently isolated at the Alfred Wegener Institute (AWI) in Germany. They present a chemolithotrophic, anaerobic methabolism and are methane producers. In addition, they have proven to be remarkably resistant against desiccation, osmotic stress, extremely low temperatures and starvation. Preliminary studies show that these archaea are able to survive simulated thermo-physical Martian conditions as well as the presence of UV-C and ionizing radiation. These features support that the methanogenic archea from Siberian permafrost are strong candidates for potential present/past life in the Martian subsurface.
The ExoMars Missions planned for 2016 and 2018 will include a Raman spectroscope among the analytical instruments. Therefore, it is very relevant to get a deeper insight in the Raman signatures of the terrestrial methanogenic archaea to better interpret the future data from Mars. As part of the "Biology and Mars Experiment" (BIOMEX) project, biosignatures of methanogenic archaea from Siberian permafrost are being studied using a novel approach of Raman spectroscopy, never used before to describe biosignatures. Using a Raman source of 533nm, interesting spectra was obtained for different species of methanogenic archaea, showing common peaks to all the studied species (around 2900nm) and other peaks of more

specific nature. Lipid fractions from soil extracts were also analysed, presenting similar (although not identical) spectra to the studied methanogenic archaea. This fact points out to some archaeal lipids, such as archaeol, as possible biosignatures.

OTP098

Novel fungal components for biofilm manipulation

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Biofouling presents a complex and a general problem in water-based industrial applications. For example, the bacterial attachment and subsequent biofilm growth on reverse osmosis (RO) membranes are largely responsible for the decline of the functional efficiency and the cost-effectiveness. To date, for membrane cleaning mechanical or chemical processes are commonly used. However, due to these treatments the membranes are often damaged which ultimately shortens the membrane life time. Therefore, several fungal supernatants are tested for active components to achieve a careful and effective biofilm detachment or destabilization from RO membranes. Fungi naturally produce a large number of metabolic products like exoenzymes. The fungal supernatants, produced by fermentation, are provided from an industrial type collection.
To find novel fungal components 406 fungal supernatants were screened in a static high-throughput crystal violet assay with biofilms of single bacterial species. The promising supernatants were subsequently characterized with further methods, such as colorimetric assays and immunofluorescence microscopy. To perform the testing of the promising supernatants closer to natural and technical environments, a microfluidic high-throughput biofilm reactor will be developed and characterized. Denaturing gradient gel electrophoresis (DGGE) was used in order to analyze the bacterial population of natural biofilms growing on RO membranes.

OTP099

Development of a clean deletion and a transposon mutagenesis procedure for *Bacillus licheniformis*

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Bacillus licheniformis is an organism of great biotechnological potential. Based on its genome sequence a directed mutagenesis protocol enables investigation of specific genes identified by sequence analysis whereas random mutagenesis is used for identification of unknown genes belonging to a defined function.
For directed mutagenesis we established and developed a markerless deletion system in *B. licheniformis*. The resulting pKVM vector series can be transferred by conjugation from *E. coli* and enables the construction of deletions up to 45 kbp. For a further improved and rapid procedure we used a nucleotide analogon for counter-selection without previous modification of the initial strain. The pKVM vectors were exemplarily used for deletion of genes involved in C2 metabolism and methylcitrate cycle.
For undirected mutagenesis we used the mariner based transposon TnYLB-1 which integrates at TA sites in the genome of *B. licheniformis*. The transposon system was transferred in a vector system capable for conjugative transfer and was subsequently used for construction of a random transposon library. Transposition-rates up to 37 % were detectable. Transposon insertion sites were identified by vectorette-PCR and inverse-PCR. Finally, the library was screened for candidates involved in anaerobic growth and utilization of acetate.

OTP100

Unusual membrane dynamics of *Ignicoccus*: 3D ultrastructure analyzed by serial sectioning and electron tomography

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The hyperthermophilic chemolithoautotrophic Crenarchaeon *Ignicoccus hospitalisis* an extraordinary organism concerning physiological features (e.g. CO_2 fixation), its ability to serve as host for *Nanoarchaeum equitans*, and also its ultrastructure [1, 2, 3]. In addition to its cytoplasmic membrane, *I. hospitalis* has an outer membrane, and, in between both membranes, a large interspace with round and elongated membrane-surrounded vesicles and tubes [1]. We are interested in analyzing the structure and network of the vesicles, the unusual overall cell architecture of *Ignicoccus hospitalis*, and the contact site to *N. equitans*, by 3D electron microscopy.
Cells were cultivated in cellulose capillaries, high-pressure frozen, freeze-substituted and resin embedded. Serial 50 nm sections were imaged by transmission electron microscopy, and data aligned and visualized as 3D stacks. For obtaining a higher resolution in the z-axis, 200 nm sections were analyzed by electron tomography. The final models show that the membrane system of *I. hospitalisis* dynamic and complex: The cytoplasmic membrane frequently forms offshoots and invaginations. Vesicles can be found that are released from or fuse with the cytoplasmic membrane; these are either free or interconnected to other vesicles. The physiological role of this membrane vesicle system is yet unknown; however, it resembles the eukaryotic counterpart (like ER, Golgi apparatus, TGN), in structure and dynamics. In addition, the *I. hospitalis* genome harbors seven proteins that are homologues to the Bet3 subunit of the eukaryotic vesicle tethering complex TRAPP I [4].
Several macromolecules are part of the contact site: The *N. equitans* S-layer, and both, the inner and outer membrane of *I. hospitalis*. According to labeling studies, *N. equitans* gains membrane lipids and amino acids from its host. 2D and 3D immuno-localisation showed that the Ihomp1 protein, the sulfur-H_2:oxidoreductase, and the A_1A_0ATP synthase are located in the outer membrane of *I. hospitalis* [5, 6], and are also part of the contact site. Biochemical studies helped to identify further proteins which might be relevant for cell-cell interaction and/or metabolite transport, like components of ABC transporters [7]. They are in the focus of ongoing studies on the contact site.

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[5] T. Burghardt et al., *Mol Microbiol* 63 (2007) 166
[6] U. Küper et al., *PNAS* 107 (2010), 3152
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OTP101

Characterisation of heat resistant spore formers isolated from foods

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Aerobic spore formers (in many cases *Bacillus* species) are consistently detected in sterilized food and display a real hazard for the food industry and the consumer. Especially dairy products like UHT-cream, UHT-milk, soft cheese or milk powder are often contaminated. In the context of a FEI/AiF research project (AiF 16012N) spore formers were isolated out of different foods (raw materials, pre and final products) and the food processing environment. In total, 450 isolates were identified via FTIR-spectroscopy or 16S-rRNA sequencing and the heat resistance of the spores was tested at 100°C for 20 min. It turned out that 97 of the 450 isolates survived this thermal treatment. 29% of these heat resistant isolates were *Bacillus subtilis*, 17% *Geobacillus stearothermophilus*, 10% *Bacillus amyloliquefaciens* and 10% *Bacillus licheniformis*. *B. subtilis* was the most frequently detected heat resistant species. The heat resistance properties of these isolates were determined in more detail and thermal inactivation kinetics of 24 different *B. subtilis* strains at 95°C and 100°C were performed. The resulting D-values were strain-specific and ranged from 15 min to more than 180 min. Further genetic and phenotypic analyses may provide new insights into the strongly varying heat resistance properties of the *Bacillus* species [1].

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OTP102

Thauera aromatica harbours a broad-host plasmid of the IncP-1 plasmid that can be eliminated in a model constructed wetland

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The denitrifying ß-proteobacterium, *Thauera aromatica*, has served as a model organism for biochemical research on anaerobic degradation of aromatic compounds for many years. The genome of the type strain, *T. aromatica* DSM6984, has now been sequenced by us. As expected, those transformation capabilities are reflected in the 4.3 Mbp genome. Unexpected, however, was the presence of a novel broad host plasmid of the IncP-1 family, pKJK10, that confers resistance to several classes of widely used antibiotics. Plasmids of this particular family are being considered as major vehicles for the spread of antibiotic resistance genes in clinical settings, thereby having a dramatic effect on medical treatment options of microbial infections. To our knowledge, this is the first time that an IncP-1 plasmid has been found in an environmental microbe. We

characterised this plasmid in detail and could show that transfer via conjugation to members of other microbial phyla occurs at high frequency under defined laboratory conditions. In contrast to those conjugation activities, in a model constructed wetland the plasmid was rapidly eliminated. These data indicate that constructed wetlands might be effective and cost-efficient means for treatment of waters rich in bacteria harbouring antibiotic resistance genes.

OTP103

Towards the understanding of the biosynthesis of β-N-methylaminoalanine in cyanobacteria

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Diverse species of free living as well as symbiotic cyanobacteria have been reported to produce the non-proteinogenic amino acid β-N-methylaminoalanine (BMAA) (Cox et al. 2005). BMAA is an environmental toxin accumulating via aquatic as well as terrestrial trophic webs and it might be involved in the etiology of motor neuron diseases in humans, like amyotrophic lateral sclerosis-parkinsonism dementia complex (ALS-PDC) (Ince and Codd, 2005). Although toxic effects of BMAA have been studied in mammalian test models and zebrafish as aquatic test model (Karaman and Speth, 2008; Purdie et al. 2009), until now no biosynthesis pathway for the production is known, which is crucial to understand if BMAA is synthesized constitutively or is regulated by environmental factors like e.g. nutrient supply.
We aim at identifying the BMAA biosynthesis pathway in cyanobacteria and will here present first results on (i) detection of BMAA in different cyanobacterial strains via a two-step HPLC-analysis and (ii) a potential reaction mechanism for BMAA in the strain *Nostoc* PCC7107.

Cox et al. 2005; PNAS 102: 5074-5078
Ince and Codd, 2005; Neuropathol. Appl. Neurobiol. 31. 345-353
Karaman and Speth 2008 ; Life Sci. 82: 233-246
Purdie et al. 2009 ; Aquatic Toxicology 95 : 279-284

OTP104

Actinobacterial chromosome tethering factor

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Bacteria exhibit a high degree of intracellular organization, both in the timing of essential processes, and the placement of the chromosome and division site. The chromosome partitioning system of the rod-shaped *actinomycete*, *Corynebacterium glutamicum* consists of the Walker-type ATPase ParA, the DNA-binding protein ParB and centromere-like *parS* sites, found near the chromosomal origin of replication. Upon initiation of chromosome replication, ParB specifically binds *parS* sites of the newly replicated *oriC*. ParA is recruited to the ParB-*parS* nucleoprotein complex, providing the driving force to relocalize the replicated *oriC* to the opposite cell pole. The ParB-*oriC* complex is then stably attached to the cell pole, where it remains and the cell divides in between the segregated chromosomes. To date, polar origin tethering factors have been identified in only few bacteria. Thus, we were interested in identifying and analyzing the polar actinobacterial chromosome targeting factor. One possible candidate for tethering the chromosome to the cell poles was the DivIVA protein, which influences apical growth and cell shape in Actinobacteria. A synthetic *in vivo* approach was employed to analyse the anchoring of the ParB-*oriC* nucleoprotein complex to the cell poles via interaction with DivIVA. In this system, *E. coli* cells, which lack homologues of the Par system and DivIVA, are used as the host for expression and interaction analysis of fluorescently labeled proteins. It could be shown that DivIVA is necessary and sufficient to recruit ParB, therefore also tether the *oriC* at the cell poles. With this synthetic system, in combination with mutational analysis, the interaction sites between ParB and DivIVA could be mapped. Indeed, analysis of a ParB mutant protein in *C. glutamicum* showed reduced polar *oriC* localization. Interestingly, the tethering of the ParB-*oriC* nucleoprotein complex at the cells via interaction with DiviVA could also be demonstrated for other members of the Actinobacterium phylum, including the notorious pathogen *Mycobacterium tuberculosis* and *Streptomyces coelicolor*.

OTP105

New thermostable glycoside hydrolases derived from thermophilic bacteria of the genus *Thermus*

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Thermus spp. constitutes one of the most widely distributed genera of thermophilic bacteria. Most of the species have been isolated from hydrothermal areas. Members of this genus are Gram-negative, non-motile rods, growing aerobically and anaerobically at an optimal temperature of 60-70°C.
Different *Thermus*-strains, able to utilize complex carbon sources such as cellulose and xylan, offer potential sources for thermostable lignocelluloses-degrading enzymes for application in biorefinery. In the so called „second generation biorefinery" lignocellulosic material from agricultural or forestry residues is used. An efficient and economic process requires suitable pretreatment of this material including the enzymatic hydrolysis of lignocellulose.
Within this project several gene libraries from different *Thermus*-strains with distinct activity towards cellulose and xylan have been constructed and screened for glycoside-hydrolases. Two new β-glucosidases could be identified using *E. coli* as heterologous host. However, no activity on cellulose and xylan was observed although the *Thermus* wild-type strains showed the corresponding activity.
To circumvent problems using a mesophilic host such as *E. coli* a new two-host fosmid system for the functional screening of gene libraries in the thermophilic host *Thermus thermophilus* will be tested currently. This system offers the chance to harness new industrial relevant enzymes from thermophilic bacteria.

OTP106

LipS and LipT, two novel thermostable lipolytic enzymes derived from soil and water metagenomes

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Lipolytic enzymes, namely carboxylesterases (EC 3.1.1.1) and triacylglycerol lipases (EC 3.1.1.3), act on ester bonds and catalyze both hydrolysis and synthesis reactions on a broad spectrum of substrates at various conditions rendering them especially suitable for biotechnological applications. Some industrial production processes demand high working temperatures and thus customized biocatalysts showing a high thermostability. Most lipases used today originate from mesophilic organisms and are susceptible to thermal denaturation (Levisson et al. 2009). Very few truly thermostable lipases are known. Here we report on the identification and characterization of two novel thermostable bacterial lipases identified by using functional metagenomic screenings. The metagenomic libraries were constructed from two different long-term enrichment cultures either inoculated with heating water or soil. Cultures were maintained at 65° to 75°C for three weeks and microbial communities characterized on a phylogenetic level based on 16S rRNA. Screening of the libraries using tributyrin and pNP-substrates (C_4 and C_{12}) at temperatures between 50°C and 70°C resulted in the identification of eleven lipolytically active clones. Two clones have been studied in detail. The identified enzymes were designated LipS and LipT. Both enzymes were expressed recombinantly in *E. coli* BL21. The *lipS* gene encodes for a 30.2 kDa protein and the recombinant enzyme reveals 50% residual activity after 48 h at 70°C while the enzyme LipT (36.1 kDa) reveals 50% residual activity after 3 h at 70°C. LipS shows an optimum temperature at 70°C, LipT at 75°C. Both enzymes catalyze the hydrolysis of medium to long-chain fatty acid esters like *p*NP-laurate (C_{12}) and -myristate (C_{14}). Furthermore, both enzymes hydrolyze a number of pharmaceutically relevant chiral substrates like naproxen and ibuprofen esters. LipS acts highly specific on an ibuprofen-phenyl ester with an enantiomeric excess (ee) of 99 % for the (*R*) enantiomer. Interestingly, LipS is able to synthesize 1-propyl laurate and other long chain fatty acid esters at 70°C. The synthesis rates were similar to those of the well-known lipase CalB. Thus, this is the first example of a thermostable metagenome-derived enzyme that has comparable activities during synthesis of polymeric substances.

Levisson, M., J. van der Oost, et al. (2009). "Carboxylic ester hydrolases from hyperthermophiles."Extremophiles13(4): 567-81.

OTP107

Seroprevalence of porcine parvovirus and leptospires in wild boars in Saxony, Germany

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Many countries in the world have populations of wild boars (Sus scrofa). They are known as reservoirs for a number of viruses as well as bacteria that are transmissible to domestic animals and humans.
Due to the increased interaction between humans and wild boars (e.g. trend of migration from cities, adaption of wild animals to urban areas) the risk of infections for domesticated animals and men is getting higher.

The importance of Leptospirosis is mainly its zoonotic potential, though it can create losses in domesticated pigs as well. The organism is commonly found in bodies of water, moist soil or vegetation contaminated by the urine or tissues of infected animals. For example, swimmers can contract the disease in contaminated infected waters.
In contrast, porcine parvovirosis represents one of the most important disorders in domesticated pigs, but is not considered zoonotic. High porcine parvovirus (PPV) seroprevalences were found in wild boars in different European countries and the population of these animals is currently increasing. Therefore, wild boars may represent a threat for domesticated pigs.
In the present study, a total of 285 samples of wild boars shot in the area around Dresden, Saxony were examined on the presence of antibodies against PPV and Leptospires.
The specific antibody titres were determined for PPV by hemagglutination-inhibition test (HI) and for Leptospira spp. by microagglutination test (MAT). The MAT panel consisted of 10 serovars. In case of PPV, titres ≥ 1:40 and in case of Leptospires, titres ≥ 1:100 were considered positive.
In total, 54.4 % of the samples were positive for PPV. To our knowledge, this is the first study on PPV seroprevalence in wild boars in Germany and the results indicate the need for further investigation.
Although the examination for leptospirosis has not yet been finished, preliminary results suggest a surprisingly low prevalence of about 2.3 %, with two sera showing MAT-titres of 1:50. In a previous study of urban wild boars higher seroprevalences (18%) were found.

OTP108

Non-conventional translation initiation in bacteria

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Initiation of translation is an important step in the process of gene expression. As initiation is the rate-limiting step of translation, most regulatory mechanisms act at this step. The well-studied conventional pathway of translation initiation in bacteria relies on the interaction of a so called Shine Dalgarno (SD) motif upstream of the start codon with the anti-SD motif at 3'-end of the 16S rRNA. In addition to conventional transcripts two types of non-conventional transcripts exist in bacteria, i.e. leaderless transcripts lacking a 5'-UTR and transcripts with a 5'-UTR without a SD motif. Initiation at leaderless transcripts requires the preassembled 70S ribosome and the initiator tRNA, while the initiation mechanism for transcripts with SD-less 5'-UTRs is still unknown.
Only about 60% of all *E. coli* genes are accompanied by SD motifs and thus about 40% have non-conventional transcripts (1). About 40 genes with and without SD motifs were chosen and the 5'-ends of their transcripts were determined using 5'-RACE. None of the transcripts was leaderless, while 18 had a 5'-UTR without a SD motif. The 5'-UTRs of three SD-less transcripts and of one conventional transcript were fused to the *gusA* reporter gene. The initiation efficiencies of two of the SD-less transcripts were about 50% compared to the highly expressed conventional control transcript and even higher during growth at 20ºC, underscoring that efficient translation is possible in the absence of a SD motif.
To gain a genome-wide overview of translational efficiencies in *E. coli* translatome analyses were established, i.e. the separation of ribosome-free untranslated transcripts and of ribosome-bound transcripts and the comparison of both fractions using DNA microarrays. As a first approach translation under standard conditions was compared to translation in the presence of Kasugamycin, which was described to specifically inhibit translation of conventional transcripts. The 5'-ends of selected transcripts were determined. In contrast to the current belief there was no correlation between the presence of an SD motif and the inhibitory effect of Kasugamycin. The next translatome analyses aim at characterizing the influence of different stress conditions on translational efficiencies and are currently under way.
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OTP109

Two new carotenoid cleavage oxygenases from marine bacteria

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Apocarotenoids are carotenoid cleavage products that are predominantly produced by carotenoid cleavage oxygenases, a class of non-heme iron enzymes that specifically cleave C-C double bonds of carotenoids. Apocarotenoids have natural functions as colorants, antioxidants, aroma compounds or hormone-like signaling molecules. They are technically applied as nutritional supplements and colorants or flavors for food, cosmetics and pharmaceutical products. To date over 2000 genomes of eukaryotes, archaea and bacteria have been sequenced and the data were published in the GenBank [1]. Numerous of these genomes contain putative carotenoid cleavage oxygenase genes that have not been investigated yet. We constructed a two-plasmid expression system for testing the carotenoid cleavage activities of such enzymes. Two carotenoid cleavage oxygenases from *Sphingopyxis alaskensis* RB2256 and *Plesiocystis pacifica* SIR-1 were further investigated.
1. D.A. Benson, I. Karsch-Mizrachi, D.J. Lipman, J. Ostell and D.L. Wheeler, Nucleic Acids Research 36 (2008), p. D25.

OTP110

Isotopic fingerprints of bacterial chemosymbiosis in the bivalve *Loripes lacteus*

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Metazoans with chemosynthetic bacterial endosymbionts are widespread in marine habitats and respective endosymbioses are known from seven recent animal phyla. However, little is known about endosymbioses in fossil settings and, hence, its ecological significance in earth history. In the presented project, we investigate the ancient and recent bivalve fauna living at marine sedimentary oxic/anoxic interfaces. Two bivalve species collected from the same benthic environment - a Mediterranean lagoon - were studied in detail. The diet of *Loripes lacteus* is based on thiotrophic gill symbionts whereas *Venerupis aureus* is a filter feeding bivalve without symbionts. The presence of three key enzymes from sulfur oxidation (APS-reductase), carbon fixation (RubisCO) and assimilation of nitrogen (glutamine synthetase [GS]) were detected by immunofluorescence in symbionts of *Loripes* and/or by activity tests in living specimens.
In search of biosignatures associated with thiotrophic chemosymbionts that might be suitable for detection of chemosymbiontic diets in recent and fossil bivalve shells, we analyzed the isotopic composition of shell lipids ($\delta^{13}C$) and the bulk organic matrix of the shell ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$). We could show that the combined $\delta^{15}N$ and $\delta^{13}C$ values from shell extracts are stable in subfossil (Pleistocene) bivalve specimens, as long as the isotopic data is "calibrated" with respective signatures from a filter feeding bivalve sampled from the same site or lithostratigraphic bed.

OTP111

A plasmid toolkit for the analysis of regulatory elements in *Bacillus licheniformis*

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Bacillus licheniformis is a valuable industrial microorganism. Strains of its species are used in industrial production of enzymes and antibiotics (1). To improve the industrial potential of this organism the investigation of the regulation, especially concerning transcriptional and translational features, is of great interest. However, the research on physiological activities during fermentation processes suffers from the lack of adequate molecular tools. Here we present a set of related *E. coli-Bacillus* shuttle vectors for the *in vivo* investigation of promoters and riboswitches. The backbone of our vectors is constructed in a modular way to ease the adaptation of the different components to a variety of experimental settings. The qualitative analysis of single cells in a culture is supported by *gfp*-vectors. The quantification of regulatory activities is the target application of our *lacZ*-vectors. The origin of replication of the pUB110 (2) vector offers the application in other species of the genus *Bacillus*. The observation that a number of strains are genetically difficult to access is addressed by the ability of the vectors to be transferred by transconjugation. We have shown that our plasmids can be transferred from *E. coli* S17-1 (3) as a donor strain to a number of test strains.
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(3) Simon, R., U. Priefer, and A. Pühler., Bio-Technology, 1983. 9(1): p. 784-791

OTP112

Characterisation of the cambialistic quercetinase from *Streptomyces* sp. FLA

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Quercetinases catalyse the 2,4-dioxygenolytic cleavage of quercetin (3,5,7,3′,4′-pentahydroxyflavone), a wide-spread plant flavonol. They belong to the cupin superfamily, which is characterised by a six-stranded β-barrel fold and conserved amino acid motifs that provide the 3-His-1-Glu ligands to a divalent metal ion [1]. Whereas many cupin-type dioxygenases use Fe^{2+} for catalysis, quercetinase (QueD) from

Streptomyces sp. strain FLA shows the highest activity with Ni^{2+} as cofactor, followed by Co^{2+} [2, 3]. The presumed ligand residues H69, H71 and H115 of QueD were individually replaced by alanine. Whereas QueD-H69A and QueD-H115A exhibited almost the same metal occupancy as the wild type protein (about 0.8 equivalents of nickel per protein monomer), QueD-H71A contained about 0.4 equivalents of nickel per monomer, indicating that H71 is important for metal binding. Replacement of H115 had only minor effects on the activity of the enzyme, whereas substitution of H71 or H69 resulted in enzymatic inactivation. Interestingly, anoxic fluorescence titration experiments indicated that the QueD-H69A protein is still able to bind quercetin with a K_d similar to that of the wild-type enzyme, suggesting that the H69 residue is relevant in catalysis rather than substrate binding. Substrate deprotonation has been discussed as the initial reaction step catalysed by quercetinases [4]. The H69 residue may act as the general base catalyst for initial deprotonation of the metal-bound quercetin.
Preference for Ni^{2+} is extraordinary for oxygenases, raising the question of whether the metal ion has a redox role in catalysis. The quercetinase reaction has been proposed to involve single electron transfer from the flavonolate anion via the metal to dioxygen [4, 5]. However, Ni^{2+} centers in ligand environments dominated by O- and N-donors were proposed to be redox inert [6]. Construction and characterisation of a Zn^{2+} isoform of QueD ion could shed light upon the question of whether the metal acts as an electron conduit. Since *in vivo* incorporation of Zn^{2+} into cytosolic recombinant QueD failed, periplasmic expression and *in vitro* transcription/translation studies are currently being performed.
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OTP113

Unravelling the role of small non-coding RNAs inMethanosarcina mazeiGö1

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In recent years the global impact of small non-coding RNAs (sRNA) in all domains of life comes more and more obvious. As still little is known on regulatory roles of sRNAs in the domain of Archaea, we recently performed a genome-wide RNA-seq approach, resulting in the discovery of 248 sRNAs in Methanosarcina mazeistrain Gö1 [1]. The archaeal model organism M. mazeiis a representative methylotrophic archaeon of significant ecological importance due to its role in biogenic methane production in various anaerobic habitats on Earth and is able to fix molecular nitrogen. Here we present the characterization of one selected sRNA, $sRNA_{162}$, using biochemical and genetic approaches. The respective results will be discussed in order to elucidate the potential regulatory role of $sRNA_{162}$ inM. mazei.
1. Jäger D , Sharma CM , Thomsen J, Ehlers C, Vogel J, Schmitz RA (2009) Deep sequencing analysis of the Methanosarcina mazei Gö1 transcriptome in response to nitrogen availability. PNAS. 106(51):21878-21882

OTP115

Changes in the microbial community structure of a fjord as a result of ecologically engineered oxygenation (Byfjorden, western Sweden)

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The availability of oxygen has a high influence on the diversity of communities and the distribution of organisms in pelagic ecosystems. Hypoxic or anoxic conditions caused e.g. by stratification lead to reduced habitats for oxygen depending eukaryotic and prokaryotic life. In recent years, oxygen depleted bodies of water are becoming more common. It is expected that in the near future anthropogenic influences like e.g. climate change and agriculture will intensify this problem. Recently, more effort has been put into the restoration of hypoxic habitats. TheBaltic deep-water OXygenation(BOX) project proposed to introduce oxygen into the long-term hypoxic or anoxic bottom waters of the Baltic Sea by using wind driven pumps to generate artificial mixing.
The Swedish Byfjorden is a long-term stratified system with a lower water column and benthic zone that has been anoxic for a long time. In addition, an inflow of freshwater from a river is generating a brackish, well-oxygenated layer of surface water with lower salinity than the deeper layers, strengthening the stratification. Because of this, the Byfjorden is an ideal model system for the Baltic Sea. As a part of the BOX project, a pilot study to test the artificial oxygenation was started in 2009. A pump was installed in the Byfjorden to mix the surface water into the deeper layers and thereby oxygenate the anoxic zone.
In this study, we monitored changes in microbial community structure in response to the oxygenation project in the Byfjorden. We analyzed water column samples from before and during the oxygenation as well as from a control station in a nearby, natural oxic fjord using a molecular microbial community profiling method. Here, we present the results in the context of biogeochemical and hydrographical data to show the impact of the oxygenation on the bacterial and archaeal community structures.

OTP116

Gene cluster for biosynthesis of the catechol-peptide siderophore griseobactin in *Streptomyces griseus*

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Iron is an essential element for the growth and proliferation of nearly all microorganisms. In the presence of oxygen, soluble ferrous iron is readily oxidized to its ferric form, which is predominantly insoluble at neutral pH. To overcome iron limitation, many bacteria synthesize and secrete low-molecular-weight, high-affinity ferric iron chelators, called siderophores, which are actively taken up as a complex with Fe^{3+} by a cognate ABC transport system. The main siderophores produced by streptomycetes are desferrioxamines.
Here we show that several *Streptomyces griseus* strains, in addition, synthesize a hitherto unknown siderophore with a catechol-peptide structure, which we named griseobactin. The production is repressed by iron. We sequenced a 26-kb DNA region comprising a siderophore biosynthetic gene cluster encoding proteins similar to DhbABCEFG, which are involved in the biosynthesis of 2,3-dihydroxybenzoate (DHBA) and in the incorporation of DHBA into siderophores via a nonribosomal peptide synthetase. Adjacent to the biosynthesis genes are genes that encode proteins for the secretion, uptake, and degradation of siderophores. Knockout mutagenesis, complementation and heterologous expression confirmed the requirement of the *dhb* genes for synthesis and secretion of DHBA and of the entire biosynthesis gene cluster for biosynthesis and secretion of griseobactin. Griseobactin was purified and characterized; its structure is consistent with a cyclic and, to a lesser extent, linear form of the trimeric ester of 2,3-dihydroxybenzoyl-arginyl-threonine complexed with aluminum under iron-limiting conditions. This is the first report on the identification of the genes responsible for DHBA and catechol siderophore biosynthesis in *Streptomyces*.
Patzer S. I., Braun V. (2010) J. Bacteriol. 192:426-35

OTP117

Biochemical and genetic characterization of ethylene glycol metabolism in *Pseudomonas putida* KT2440 and JM37

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Being an important building block for flavor chemicals and polymers, glyoxylic acid is a valuable product for many industrial processes. The enzymatic oxidation of ethylene glycol could provide an interesting alternative to the commonly used chemical synthesis of glyoxylic acid. In order to develop such a biocatalyst, we started to investigate the metabolism of ethylene glycol using the *Pseudomonas putida* strains KT2440 and JM37.
We found that *P. putida* JM37 rapidly grows in minimal media containing ethylene glycol or glyoxylic acid as sole source of carbon and energy, while strain KT2440 did not show growth even after three days of incubation. However, experiments with dense cell suspensions revealed complete conversion of ethylene glycol for both strains. In contrast to JM37, strain KT2440 showed temporal accumulation of glycolic acid and glyoxylic acid as intermediates, finally yielding oxalic acid as the end product.
To identify key enzymes involved in the metabolism of ethylene glycol, a differential proteomic approach was used. Increased expression of tartronate semialdehyde synthase (Gcl), malate synthase (GlcB), and isocitrate lyase (AceA) in strain JM37 as well as AceA in strain KT2440 was found during incubations with ethylene glycol or glyoxylic acid. A corresponding triple mutant strain harboring an additional deletion in *prpB*, encoding for methyl isocitrate lyase, was constructed and characterized in strain KT2440. This mutant showed a significant reduction in the conversion of ethylene glycol and increased accumulation of glycolic acid and glyoxylic acid compared to the wildtype strain. Further analysis uncovered the induction of two PQQ-dependant ethanol

dehydrogenases, indicating their important role within the oxidative metabolism of ethylene glycol. This hypothesis was further supported by a corresponding double deletionmutant, which shows a strong decrease in ethylene glycol metabolism.

OTP118

Subtyping off17- related genes in wastewater of slaughterhouses

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The zoonotic pathogens ofE.colican survive over long periods in sewage sludge as well as on pasture land and in association water systems. They could be widely spread in the environment by direct land application of sludge or by regular contamination of surface water, but limited information is available concerning the spreading of these pathogens in sewage of slaughterhouses. TheF17family includesF17a, F17b, F17c, F111fimbriae produced by bovineE.colistrains. Wastewater samples from 12 slaughterhouses located in different regions inFrancewere tested to detect theF17-related fimbriae and to detect four subtypes of structural subunit genes positiveEscherichia coliisolates. A total of 224 wastewater samples were collected in wastewater treatment plants at different stages of wastewater processing in small and big abattoirs and down and upstream rivers, screened for the presence ofF17 genes(F17 a- A gene, F17 b- A gene, F17c-A/gafA gene and F111-A gene) by multiplex PCR.F17positiveE. coliisolates were detected in 24 % of the samples collected (54/224); F17 a- A gene were found in 18 %,F17 b- Agene in 4%,F17c-A/gafAgene in 41% andF111-Agene in 37% of the samples in all slaughterhouses, respectively, suggesting that they could be spread into the environment. Our results suggest that the diversity of theE. coli-associated virulence factors in the strains indicates that the environment may play an important role in the emergence of new pathogenicE. colistrains and to increase our knowledge of the important prevention needed in our environment from the pathogenicE. coliand their mutual correlation.

Keywords: slaughterhouses- wastewater-multiplex PCR-F17 a- A gene, F17 b- A gene, F17c-A/gafA gene and F111-A gene.

OTP119

Identification of Lignin-degrading enzymes from bacteria

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Lignin is the most abundant renewable source of aromatics.[1] Therefore, it is an interesting natural source for aromatic compounds in chemical synthesis, glues or biobased materials. Lignin, together with Cellulose and Hemicellulose, is part of the structural framework in plants.[2] There are huge amounts of lignin available from straw, waste wood or by-products from paper industry (~50 mio t/a). Nowadays around 98% are burned to produce energy. A prerequisite for the efficient utilization of lignin as a resource for chemicals is an adequate depolymerization process to obtain aromatic monomers from the recalcitrant polymer structure. Several enzymes have been shown to be involved in the enzymatic lignin degradation process, especially laccases (EC 1.10.3.2) and peroxidases (lignin peroxidase, EC 1.11.1.14 and manganese peroxidase,EC 1.11.1.13) from white-rot fungi.[3]

Since the commercially available lignin-modifying enzymes (LMEs) from fungi are too expensive for the use in industrial applications, we are focussing on the identification and overproduction of LME from bacteria. To our knowledge, no lignin degrading enzymes from bacteria are commercially available. We have identified seven bacterial strains with lignin-degrading potential from the literature and conducted cultivation experiments to determine LME activity in the culture supernatants. Two media with or without lignin as inductor have been used. Our results show that six strains grew in the culture media supplemented with 0.2% (w/v) lignin. Meanwhile one strain was able to grow with 0.1% (w/v) but not with 0.2% (w/v) lignin. As expected, all of the strains were able to grow in the standard media without lignin. In most of the bacteria a significant increase in LME production was determined when supplemented with lignin. Currently, genomic libraries of selected bacteria out of these seven strains are constructed and LMEs will be identified applying high-throughput screening (HTS) methods.

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OTP120

Beat the cold: Multiple roles of the RNA helicase CshA at lower temperatures in *Bacillus subtilis*

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In its natural habitat, the upper layers of the soil, *Bacillus subtilis* has to cope with a wide range of environmental challenges like low temperatures. Under these conditions the bacterium is faced with decreased membrane fluidity and changes in the topology of the DNA. Furthermore, the formation of secondary structures of RNA is favored with decreasing temperatures. To avoid undesirable intra- and intermolecular interactions of RNA molecules, the cell encodes a variety of proteins helping the RNA to fold properly. One of the largest protein classes in RNA metabolism are DEAD-box RNA helicases. Such RNA helicases are highly conserved enzymes utilizing ATP to bind and remodel RNA or ribonucleoprotein complexes.

In this study we have investigated the impact of the DEAD-box RNA helicase CshA on the growth of *B. subtilis* at low temperatures. We show that under these conditions CshA is crucial for the bacterium to survive and deletion of *cshA* leads to aberrant cell morphologies. Using a wide set of experiments we demonstrate that CshA is involved in the degradation of mRNA, the proper assembly of ribosomes and interactions with proteins of the cold shock response. Taken together, the DEAD-box RNA helicase CshA has multiple roles in the adaption process of the cell to lower temperatures thereby dealing with rRNA and mRNA molecules.

OTP121

Conjugative plasmid pLS20 of *Bacillus subtilis* alters the transcriptome and physiology of its host organism

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Bacillus subtilis strains from the environment harbor different plasmids, which have been shown to alter different physiological traits, such as biofilm formation. Here we analyse in detail the effect of a large conjugative plasmid found in *B. subtilis* isolate (*natto*) used for food processing in Japan. Our work provides evidence that the plasmid pLS20 induces a global change in gene regulation on the host chromosome, but maintains and propagates itself without harmfully burdening the host. Exponentially growing cells exhibited numerous differences in the expression of genes involved in the intermediary metabolism, cell envelope, different cellular processes, stress resistance and motility. Several changes lead to a benefit for the fitness of the host to adapt to environmental changes, the observed reduction of motility may lead to a benefit for the plasmid for more efficient transfer between bacteria. Interestingly, plasmid pLS20 shows a significantly extended lag phase compared to plasmid-free *Bacillus* cells, and conjugates most efficiently during the lag period between stationary phase and exponential growth. The later commencement of growth is accompanied by the induction of transfer genes during this growth phase, while exponential growth leads to a reduction in transcription rates of conjugative proteins. Our work reveals a mutual benefit for host and conjugative plasmid and a differentiation-like behavior of conjugative DNA transfer.

OTP122

Removal of pharmaceutical compound diclofenac by iron bacteria

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In recent years, pharmaceuticals are increasingly being detected in many waterways all over the world. As a frequently prescribed non-steroidal anti-inflammatory drug, diclofenac has been ubiquitously detected in the influents and effluents of wastewater treatment plants (WWTPs) at the µg/l level, and it also occurs at concentrations of the ng/l level in surface water, ground water and even in drinking water. Although the acute ecotoxicity of diclofenac is relatively limited, it definitely poses a risk on the ecosystems where it is present.

Biotransformation is generally considered to be the main process by which to remove pharmaceuticals, both in WWTPs and in aquatic environment. In many cases, microorganisms are applied to mineralize the pollutants to water or degrade them to acceptable forms. In this work, pure cultured iron bacteria were utilized to remove diclofenac. Meanwhile, the various factors that might affect the removal efficiency, such as initial diclofenac concentration, residual Fe^{2+} levels, and Mn content, were investigated.

The study results indicated that some strains of iron bacteria were very effective to remove diclofenac under axenic condition. Among the 18 tested strains, 4 strains showed removal efficiency above 90%.

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OTP123

Fluorescence microscopical analysis of PHB granule associated proteins (PGAPs) in *Ralstonia eutropha* H16

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Ralstonia eutropha H16 has become the model organism for studying metabolism of poly(3-hydroxybutyrate) (PHB), an important biodegradable biopolymer [1]. Despite > 2 decades of intense research on PHB metabolism new PHB granule-associated proteins were recently discovered using a two hybrid screening approach [2]. Meanwhile, at least 19 proteins are known that are important for biosynthesis, maintenance and intracellular mobilization of PHB in *R. eutropha*. These are: acetoacetyl-CoA-thiolase (PhaA) and acetoacetyl-CoA reductase (PhaB) that are necessary for synthesis of the PHB monomer (3-hydroxybutyryl-CoA), five phasinproteins (PhaPs) that include major constituents of the granules surface layer, 9 PHB depolymerases (PhaZs) (two of which are oligomer hydrolases), PHB synthase (PhaC), regulator PhaR and recently discovered PhaM that ensures equal distribution of PHB granules during cell division [3]. Many of the above-mentioned proteins presumably are attached to the PHB granules surface layer. However, only for PhaC, PhaR, PhaP1, PhaP5, PhaZa1 and PhaM data are published that confirmin vivoattachment of these PGAPs. In this study we determined subcellular localization of all currently known 5 phasin proteins (PhaP1-5) under condition permissive and restrictive for PHB accumulation. N- and C-terminal fusions of the respective phasin protein with eYfp were constructed and the respective fusions cloned on a braod host range plasmid were conjugatively transfered to *R. eutropha*. All fusions were expressed in the wild type H16, in strain PHB-4 (a chemically induced mutant with a nonsense mutation in PhaC) and in a chromosomal Δ*phaC* mutant. Similar fusions were constructed for all putative PHB depolymerases. The results for the depolymerases will be presented in a separated poster (A. Sznajder et al.).

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OTP124

Elucidating the CRISPR-Cas-System of *Methanosarcina mazei* Gö1

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Methanosarcina mazei strain Gö1 (*M. mazei*) belongs to the methylotrophic methanogens of the order *Methanosarcinales*, which have the most versatile substrate spectrum within the methanogenic archaea contributing significantly to the production of the green house gas (Rogers & Whitman, 1991; Thauer, 1998).
The genome annotation published in 2002 (Deppenmeier et al., 2002) did not include the information on potential CRISPR loci in archaeal model organism. Our recent investigations however identified the presence of two main CRISPR loci in *M. mazei*. As characteristic for CRISPR loci, both of them contain a conserved direct repeat of 37 nucleotides in length. The first CRISPR locus, containing 47 direct repeats with spacers, is flanked by a Cas type I-B system, whereas the second locus (containing 81 direct repeats) is flanked by a polycistronic operon encoding a RAMP module of CAS proteins (type III-B). Interestingly, based on sequence homology of already known Cas6 proteins, none of the loci obviously encode for the major endoribonuclease of crRNA maturation. Here we present the identification of potential *M. mazei* Cas6 orthologs. The biochemical characterization of the protein(s) will be presented and discussed.

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OTP125

SMC is recruited to *oriC* by ParB and promotes chromosome segregation in *Streptococcus pneumoniae* and *Bacillus subtilis*

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Reliable segregation of replicated chromosomes is a prerequisite for maintaining genomic integrity. Multi-protein complexes formed by the Structural Maintenance of Chromosomes (SMC) proteins are essential players for performing this task both in mitosis and meiosis, as well as during the bacterial cell cycle.
SMC proteins are highly conserved in all domains of life. Most bacteria express a single SMC that is associated with the kleisin ScpA and ScpB protein to form a complex called "bacterial condensin". In many bacterial species condensin is indispensable for proper chromosome condensation and segregation.
We found that condensins of Bacillus subtilis and the human pathogen *Streptococcus pneumoniae* promote segregation of replicated chromosomes and are recruited to *parS* sites at the origin of replication by the sequence specific DNA binding protein ParB. This targeting mechanism seems to be conserved at least among gram-positive bacteria and can be reconstituted in a heterologous expression system.

OTP126

Bacterial cytoskeletal element MreB forms dynamic actin-like filaments in live cells and in vitro

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MreB protein is an essential component of the cell shape generation system and additionally affects many subcellular positioning processes in bacteria. MreB has a three dimensional structure that is highly similar to that of actin and forms filamentous structures in vitro. However, it has still been a matter of dispute if actin and MreB have arisen through divergent or convergent evolution. It has recently been proposed that the activity of MreB does not depend on the formation of extended filaments and that the protein forms patch like structures rather than dynamic filaments in vivo. Using super resolution microscopy (S-TIRF) with a resolution of 100 nm, we provide evidence that MreB forms filaments in live Bacillus subtilis bacteria, which can extend at a rate of 65 nm/s and mostly have a length in between half and full turns around the cell periphery. Filaments display a surprisingly variable degree of orientations, from circumferential to helical, can fuse and split, and show extension dynamics that are affected through a point mutation within the ATPase motif. FRAP experiments reveal very fast exchange rates consistent with rapid filament turnover. MreB and its three paralogs Mbl and MreBH also form polymers in vitro, dependent on ATP and magnesium. Our results demonstrate that MreB forms extended filamentous structures that are able to confer long range interactions with membrane proteins, which can be circumferential as well as helical. Given that any polymer has an inherent bending stiffness, and that MreB filaments are mostly longer than a half turn around the cell periphery, filaments may exert a mechanical force against the membrane that can lead to local transfer of energy against the wall, possibly facilitating the incorporation of new peptidoglycan strands into the existing wall polymer. Our data further support the notion that MreB and actin have had a common ancestor whose function was already based on dynamic filament extension/retraction reactions.

OTP127

Analysis of the complete genome of *Janthinobacterium* sp. HH01 reveals a homoserine lactone-independent regulation of the violacein biosynthesis genes

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The gram-negative β-proteobacterium *Janthinobacterium* sp. HH01 was recently isolated from an aquatic environment. *Janthinobacteria* form beneficial biofilms on the skin of amphibia and are involved in preventing fungal growth [1,2]. HH01 grows well in a wide temperature range between 4 and 17 °C and produces violacein in stationary growth phase.

In order to analyze the role of HH01 and its relation to the eukaryotic host, we established its genome sequence. The genome was determined with a size of seven Mb. The most important finding was a number of PKS/NRPS-gene clusters that make this microbe potentially interesting for the synthesis of novel drug molecules. In addition, it revealed the presence of all known secretion systems (except type III) and a violacein biosynthesis operon. But contrary to the AHL (acyl homoserine lactone)-dependent regulation of the known *Chromobacterium violaceum* violacein biosynthesis operon [3], no evidence for an AHL-dependent mechanism was observed.
To identify structural and regulating genes linked to the violacein synthesis a Tn5 transposon mutant library of about 7,000 clones was screened for clones impaired in violacein biosynthesis. About fifty white or weakly violet mutant clones were obtained and subsequently analyzed by PCR and complementation experiments. Besides a number of mutations located in structural genes, several mutations could be linked to the regulatory pathway associated with the violacein gene expression. These mutants are currently being investigated in more detail to elucidate the quorum-sensing system of this newly discovered organism. Moreover data from the genome annotation and complementation tests suggests that HH01 controls the violacein biosynthesis using a single autoinducer synthase and corresponding receptor similar to the *Vibrio cholerae* CqsA/CqsS quorum-sensing system. Thus this is first example of a violacein biosynthesis pathway that is not controlled by the influence of AHL autoinducer molecules.
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OTP128
L-lysine production by *Corynebacterium glutamicum* utilizing alternative renewable resources
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With a world market of 1.5 Million tons per year, the essential amino acid L-lysine is one of the most important biotechnological products. L-lysine is mainly produced by fermentation using sugar-based feedstocks consisting of glucose, fructose or sucrose. However the use of these sugars has certain disadvantages. On the one hand the prices are constantly rising and on the other hand the use of sugars competes with the food industries. Therefore alternative carbon sources like lactate [1] or xylose [2] gain interest for fermentative production. Systems metabolic engineering provides an excellent starting point to establish corresponding production processes. Recently a superior genetically defined *Corynebacterium glutamicum* strain was created with excellent production properties during growth on glucose and industrially relevant feedstocks including molasses and corn steep liquor [3].
In this work we investigated the use of lactate and xylose as alternative carbon sources. For xylose this first required metabolic engineering of the xylose assimilation pathways as previously demonstrated for the production of diaminopentane [2].
Lysine production from lactate also requires engineering of *C. glutamicum*. Consequently the L-lysine hyper producing *C. glutamicum* strain was modified to better growth on lactate. This included overexpression of the gene for quinone-dependent L-lactate dehydrogenase (LldD), by using a native strong promoter.
Both producer strains were investigated for their performance to produce lysine. They exhibited interesting properties and serve as a valuable proof of concept for bio based production on novel feedstocks.
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OTP129
MenD- a biocatalyst for asymmetric C-C-ligations
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Besides their natural functions in cell metabolism, many thiamine diphosphate (ThDP) dependent enzymes can be used *in vitro* for C-C-ligation reactions (pyruvate decarboxylase, transketolase, benzoylformate decarboxylase and others).[1] In menaquinone biosynthesis, the ThDP-dependent MenD (2-succinyl-5-enol-pyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate (SEPHCHC) Synthase) performs a Stetter-like 1,4 addition reaction by ligating isochorismate and 2-ketoglutarate under non-oxidative decarboxylation to yield SEPHCHC.[2,3] MenD (subunit size of 65 kDa) from *E. coli* requires ThDP and a bivalent metal ion.[4] So far, MenD is the only described enzyme catalyzing a Stetter-like reaction which means that a nucleophilic donor substrate (2-ketoglutarate) is attached to α,β-unsaturated carbonyl acceptor (e.g. isochorismate). The reported K_M and K_{cat} values are in the μM and 1 min^{-1} range, respectively.[3] Crystal structures for MenD wild type and mutant proteins have been reported from *E. coli* and *B.subtilis* allowing insights into amino acid residues of the active site which are discussed for cofactor and substrate binding. [4,5,6]
Besides the Stetter-like reaction, MenD also catalyzes an 1,2-addition and provides the approach to hydroxyketones.[7] Therefore, it is a promising biocatalyst for C-C bond formation, but the substrate range has to be extended (e.g. 2,3-CHA) to gain access to new products. Also, *in vivo* biosynthesis of MenD products with recombinant *E.coli* cells is a goal. Work on both the clarification of structure-function-relationship and the extension of the substrate range is underway and data will be presented.
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OTP130
Phototrophic microbial fuel cell: Mircobial ecology for electroactive systems
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One century ago (1911) Potter has shown that electricity could be generated by anaerobic microbial respiration of organic matter: the first microbial fuel cell (MFC) was born. MFC systems are composed of two compartments: an anodic chamber and a cathodic one. The electricity is generated in the anodic side by the microbiologically driven transfer of electrons from secondary fermentation products (e.g: fatty acid, ethanol, lactate, butyrate, acetate etc) to the electrode. The anode and cathode electrodes are connected through a circuit, which facilitates the flow of electrons from the former to the latter; this results in the production of electrical current. The produced protons (H^+) diffuse from the anodic compartment into the cathodic chamber, through a proton exchange membrane (PEM) where they react with oxygen and incoming electrons, thus producing water.
The project aims are to use MFC for both carbon capture and electricity production. To achieve our objectives, we reproduce a controlled electron cascade such as the one occurring in stratified microbial food-web (e.g. microbial mats). In such systems, carbon is fixed through photosynthesis as biomass that is further consumed by underlying anaerobic respirations. In our case, we cultivate oxygenic phototrophs for their capacity of extracting electrons from water and utilize it to reduce inorganic carbon into biomass. In addition, the by-product of their metabolism, oxygen, will enhance cathode efficiency by an O_2 supersaturation effect. The produced biomass will therefore serve as the electron donor for anaerobic respiration in the anodic compartment. In this system the inorganic carbon serves as a transporter to harvest light energy in the cathodic compartment and to release it in the anodic one as electrons. The main challenge is to control the recycling of elements between those two compartments in order to be as close to as possible a semi-closed artificial ecosystem. Therefore, we will obtain a carbon-neutral electroactive system. However, as we are reproducing an artificial microbial ecosystem, certain ecological concepts have to be taken into account. Thus, we will present the ecological aspects that we have to control in order to produce an electroactive and carbon-neutral semi-closed system.

OTP131
Lake La Cruz, a Neoarchean Ecotone Ocean analogue
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Recent research on the biogeochemistry of the Late Archean Ocean (2.7-2.5 Ga) showed that there was a spatial patchwork of physical-chemical conditions. A simplified model of an Archean Ocean would thus consist of two distinct compartments with different dominating biogeochemical processes: I) a shallow Ocean Margin compartment with oxygenic photosynthesis in the upper water column, and euxinic conditions (anoxic and sulfidic) below the chemocline and in the sediments; II) an anoxic Fe(II)-rich Open Ocean compartment with a primary production dominated by anoxygenic photoferrotrophy and methanogenesis prevailing organic matter degradation in bottom layers and sediments.
Whilst analogues of an ocean with established sulfur cycle, corresponding to compartment I, have been described and well studied (e.g. Black Sea or Lake Cadagno), only one modern analogues of the ferruginous open water compartment has been described. Therefore, we study the microbial iron cycling, in the ferruginous water column of Lake La Cruz (Spain). We

have shown direct evidences, for the first time, of the activity of anaerobic ferro-oxidizers within the water column. We have demonstrated that those autotrophs thrived by photoferrotrophy (anoxygenic photosynthesis) or by chemo-ferrotrophy (nitrate-dependent respiration). More over, all the metabolisms thought to have existed in the Late Archean Ocean are present in Lake La Cruz. Therefore, the results from this study combined with those of previous studies allowed us to establish a biogeochemical model that complement the ones describe above. Accordingly, the water column of Lake La Cruz may represent an ecotone between the two main Neoarchean Ocean compartments and, consequently, be a good model system, or samples source, for studying metabolic activity interactions in experimental conditions that reflect theoretical models of the Archean Ocean.

OTP132

Elimination of indicator bacteria and viruses in open and covered simulation channels of streaming water

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Indicator bacteria have been shown to have limited value as indicators for the presence of viruses in water systems. For this reason, bacteriophages (coliphages) or human adenoviruses have been proposed as additional indicators for human pathogenic viruses. To test their suitability, survival of indicator bacteria and viruses after release from a waste water discharge into a river was studied in a river water simulation plant. The plant consisted of elliptical channels each of 100 m length, 100 cm diameter and 40 cm water depth, allowing online monitoring of selected physical and chemical parameters. Concentrations of *E. coli* and intestinal enterococci, as well as somatic coliphages, F+phages, human adenoviruses and noroviruses were monitored after a peak contamination of channel water with 1% or 5% of primary waste water effluent. Special attention was given to the effect of sunlight and its UV components on the survival of these bacteria and viruses by using an open channel and a channel protected from sunlight at different times of the year. Radiation was monitored as mJ/cm^2 from meteorological data. The disinfection effect of radiation was characterized through time and dose dependend kinetics of elimination of test organisms as log unit per time (λ_t) or per radiation dose of sunlight (λ_d). As expected, the effect of solar radiation was dependent on the season with maximum effects during summer. The effect of radiation differed with regard to the test bacteria and viruses. Exposure to sunlight had a more significant effect on the indicator bacteria than on the viruses. Most prolonged survival was found for somatic coliphages as well as for human adeno- and noroviruses. These results support previous findings that indicator bacteria are no good indicators for viruses and suggest coliphages as suitable viral indicators, especially under adverse environmental conditions like high irradiation intensity.

OTP133

Microvirin - a novel cyanobacterial lectin with broad and potent anti-HIV activity

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Carbohydrate binding agents such as lectins have proven to be valuable source of anti-HIV therapeutics that may be applied as microbicides. It is known that a variety of mannose-specific plant lectins that bind oligomannose glycans have strongin vitroanti- HIV-1 inhibitory activities, and therefore have been proposed as microbicide candidates for topical prophylaxis of HIV-1 infection and as potential anti-HIV therapeutics.
Here, we present the mannan-binding lectin microvirin (MVN) from the cyanobacterium *Microcystis aeruginosa* PCC 7806 that represents a promising new HIV microbicide candidate. The sugar specificity of the protein was elucidated through carbohydrate microarrays, which revealed MVN to be selective for mannan-type oligosaccharides with terminal a(1-2)-mannose moieties. Compared to the related protein cyanovirin-n it exhibited comparable and broad anti-HIV-1 activity against all evaluated HIV-1 virus strains and clinical isolates, but was much less (>500-fold) cytotoxic when evaluated in various human T cell lines and human peripheral blood mononuclear cells (PBMC). In addition MVN was not mitogenic, did not induce cellular activation markers in PBMC and never enhanced viral replication, as this was observed with cyanovirin in specifically designed PBMC assays. The possible pathogenic consequences associated with these side-effects have now raised the issue of safety of all other members of the antiviral class of lectins. We so far conclude that MVN has a superior safety profile in comparison with other members of the antiviral lectins that have been proposed as microbicide candidates such as cyanovirin-n.
Apart from its antiviral potential thein vivofunction of MVN was extensively studied. Immunofluorescence microscopy (IFM) as well as lectin binding analysis (LBA) using FITC-labelled MVN were employed and confirmed that MVN is secreted from *M. aeruginosa* cells and binds to LPS on its cell surface. *M. aeruginosa* cells form large colonies and MVN is proposed to be involved in the cell-cell attachment. MVN orthologues were identified in different cyanobacterial genera and are currently cloned and heterologously expressed in order to evaluate their antiviral activity.

OTP134

Engineering of *Escherichia coli* cells for the heterologous production of fucosylated human milk oligosaccharides

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Among other biologically active substances, oligosaccharides represent a fundamental component of human milk. They are known to show beneficial effects for infants, such as inhibition of pathogenic infection by binding pathogen receptors and growth promotion of bifidobacteria as key commensals [1]. So far, investigation on the physiological function of milk oligosaccharides had only been accomplished by the use of single compounds or mixtures that were purified from breast milk. Comprehensive study or even clinical trials with single compounds isolated from human milk were not possible, because major parts of the oligosaccharides in human milk are found only in small quantities.
The work described here focuses on a novel method for the efficient synthesis of oligosaccharides. The synthesis proceeds via a glycosylation reaction in recombinant *Escherichia coli*, which expresses suitable glycosyltransferases. The activated sugar precursors that are required for glycosyltransferase catalyzed reactions are generated by the metabolism of the organism. The principle possibility for a heterologous biosynthesis of fucosylated oligosaccharides in *E. coli* was shown before [2, 3]. Here we present the construction of a plasmid-free strain for the heterologous synthesis of 2'-fucosyllactose using the λ-Red recombineering technique [4]. After optimization of the heterologous gene expression, 2'-fucosyllactose was produced in a large scale fed-batch bioreactor cultivation using glycerol as carbon source and lactose as substrate.

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OTP135

Functional expression of the dirigent protein AtDIR6 in *Pichia pastoris*

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The biosynthesis of lignans, a diverse class of secondary metabolites in plants, is initiated by an one-electron oxidation of monolignol substrates, followed by a phenoxy radical coupling reaction. In plants, this reaction can occur in an enantioselective fashion.Interestingly, oxidases such as laccases and peroxidases, which are essential to generate the initial radicals for the subsequent coupling reaction, do not exhibit any regio- or stereoselective control. The discovery of dirigent proteins fromForsythia intermedia (FiDIR1) [1] andArabidopsis thaliana(AtDIR6) [2] mediating the stereoselective 8-8´coupling of coniferyl alcohol to either (+)- and (-)-pinoresinol, respectively, uncovered the nature of such an enantioselective control. The mode of mechanism is still elusive, however, it is suggested that dirigent proteins exist as homodimers lacking oxidative capacity themselves. They rather capture and orientate free radicals generated from oxidases in such a way that a specific coupling mode is favored, leading to the formation of optically active compounds. In order to uncover the underlying mechanism of this reaction, an effective protein expression system based on a fermentation process would be highly beneficial.
Therefore, we heterologously expressed the dirigent protein AtDIR6 inEscherichia coli(E. coli) andPichia pastoris(P. pastoris). While expression inE. colidid not yield a substantial amount of soluble protein in different strains and under various conditions, fed-batch fermentation ofP. pastoris resulted in 47 mg/L of glycosylated AtDIR6, which represents a more than 300 fold increase in yield compared to the expression with plant suspension cultures. We found that the enantiomeric excess of (-)-pinoresinol in the phenoxy radical coupling of coniferyl alcohol using the purified enzymein vitrowas comparable to the plant-derived enzyme. Further, we could demonstrate that the glycosylation ofP. pastoris-derived AtDIR6 is essential for its dirigent activity. Taken together with the results obtained from CD-spectroscopy, our data strongly indicate that the glycosylation of AtDIR6 is critical for initial folding process as well as for the conformational stability of the protein.

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OTP136

The use of copper slag as armor stone in running waters - How does rock chemistry effect natural biofilm formation?

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Benthic biofilms are intimate associations of benthic algae and heterotrophic microbes within a matrix of extracellular polymeric substances. They fulfill important ecosystem functions by providing basal energy resources to higher trophic levels in lotic foodwebs and removing nutrients from the water column. They may also serve in the sequestration of pollutants such as metall(oids), however, these may re-enter the foodweb via grazing organisms. Copper slag, a by-product of ore processing, is a preferential constructing material in water ways due to its high mass density. Its use, however, may result in the release of eco-toxicologically relevant metall(oids) (e.g. Cd, Zn, Cu). Hence, the aim of a joint project with the German Federal Institute of Hydrology is to investigate the environmental impact of copper slag on benthic organisms with a special focus on the development of natural benthic biofilms in the present study. Six indoor stream mesocosms, each with a closed water circuit, were set up and filled with sediment and water (625 l) of the river Rhine. Three of the six channels contained additionally rocks of copper slag, the other three contained basalt rocks as reference material. Biofilms were sampled in 4-week intervals over a period of six months. In order to differentiate between the effects of leached (dissolved) metall(oid)s and those of the rock surface chemistry, biofilms were sampled from copper slag and basalt rocks as well as from rocks originating from river Rhine sediments. Biofilm samples will be characterized by determining total organic carbon (total biomass), Chla(autotrophic component), phospholipid-P (living biomass), and taxonomic composition. The determination of both, total and living biomass, allows us to differentiate between mere biomass accumulation and actively growing or regenerating biomass. Preliminary results will be presented and discussed against the background of metall(oid) leaching and accumulation.

OTP137

Non-standard circadian clock systems in cyanobacteria

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Many organisms adapted their biological activities to environmental changes associated with alternations of day and night. Most eukaryotes even evolved internal timing systems to predict those day-night cycles. Among prokaryotes solely cyanobacteria are known to posses such a circadian clock. In the model strain *Synechococcus elongatus* PCC 7942 it consists of just three proteins (KaiA, -B and -C) that display 24hr oscillations in protein abundance, complex formation and posttranslational modification. KaiC as the core component undergoes rhythmic autophosphorylation and -dephosphorylation. These oscillations are a consequence of KaiA sequestration by KaiC hexamers and KaiBC complexes (1).
The number of *kai*-genes, however is not conserved among cyanobacterial species. Prochlorococcus has lost the *kai* gene and harbors a less robust clockwork based on KaiB and -C (2). In contrast, *Synechocystis* sp. PCC 6803 expresses *kaiA*and even three homologs of both *kaiB* and-*C*.
To gain insights into the non-standard circadian clock of *Synechocystis* we are characterizing its multiple Kai proteins *in vitro* and *in vivo*. Our *in vitro* data suggest partial differences in their biochemical properties. Comparable to the well-studied *Synechococcus* counterpart, autophosphorylation of KaiC1 is enhanced by KaiA1, whereas the kinaseactivity of KaiC3 is independent of KaiA1. For *in vivo* analyses specific antibodies against KaiA and the different KaiC proteins are available allowing us to investigate the putative dynamic behavior of the *Synechocystis* Kai proteins under different light/dark cycles as well as under continuous conditions.
Our findings suggest that the clockworks of cyanobacterial timing systems do not follow a universal blueprint. Further analyses will gain insights how the composition of these clockworks contributes to their precision and robustness. Additionally our results might provide implications for the putative timing mechanisms of other bacterial species, such as purple bacteria, which encode KaiB and -C homologs but lack a *kaiA* related gene.

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OTP138

Phylogenetic analysis of β-Lactameses

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Objectives: The number of annually identified b-lactamases with extended activity against cephalosporines (ESBL) increased during the last decade indicating the need for appropriate deescalating antibiotic strategies. Thus, due to high recombinative genetic material of bacteria determination of species remains not sufficient for clinical use. Based on amino acid sequence b-lactamases exhibit different substrate pattern allowing classification into 16 functional groups [1]. Therefore, knowledge of the sequence is essential to identify ESBL variant and resistance properties. Although, some studies were performed to investigate substitutions of TEM, SHV and CTX-M, SNP determination of other b-lactamases like OXA or AmpC reminds more challenging due to the high sequence variability. Here we present an overall phylogenetic update of b-lactamases based on amino acid (aa) sequences correlated to substrate-inhibitor profiles.
Methods: We collected aa sequences of b-lactamases from NCBI database. In total, 643 sequences with at least 200 residues could be aligned and analyzed using DS Gene 1.5 software (Accelrys Ltd) with the phylogenetic method by neighbor joininging. The resulting phylogenetic tree was correlated to the functional properties proposed by Bush and Jacoby in 2010 [1] and analyzed in detail.
Results: As expected, the alignment reflected the differences of the hydrolyzing mechanisms of the β-lactamases. Closer relationships were found for AmpC and OXA-type, whereas GES, CTX-M, IMI and KPC formed another phylogenetic group. Moreover, we could point out mutation hot spots, which are responsible for specific changes of the phenotype.
Conclusion: Due to the expanding multi-resistance of pathogens, a fast identification of the ESBL-variant is in focus of clinical interest and will allow the appropriate therapeutic intervention.
We found evidence that some unique aa substitutions are sufficient to cause specific changes in the phenotype of TEM indicating that better understanding of substitution's dynamics within the types might simplify the determination of the given b-lactamase by SNP typing. We focus on the validation of such unique substitutions within the other molecular classes that exhibit much higher sequence variation compared to TEM.

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OTP139

Characterization of a Lipopeptide Biosurfactant Produced by Bacteria Isolated from Petroleum-Polluted Soil

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Biosurfactants are environmentally benign microbial products with tremendous environmental, industrial and biomedical applications. The large scale production of biosurfactants has been hampered by their high production costs, poor strain productivity and the use of expensive substrates. Here, two bacterial strains were isolated from petroleum-contaminated soil via enrichment in rich medium (LB) and minimal medium containing 2% Arabian light oil as the sole carbon source. Based on 16S rDNA genes sequencing and phylogenetic analysis, the isolated strains could be affiliated to different species of the generaBacillus(strain I-15) andPseudomonas(strain I-19). Both strains emulsified crude oil in minimal medium within 2 to 7 days of incubation at 30℃. The oil droplets of the produced emulsions had various sizes, indicating the production of different types of biosurfactants/ bioemulsifiers. Preliminary screening assays such as oil displacement and droplet collapse, revealed the presence of extracellular surface active agents (surfactants) in the cell free culture supernatants.The I-19 isolate produced biosurfactant only when grown on hydrophobic substrates such as crude oil and diesel. Whereas the I-15 strain produced biosurfactant when grown on crude oil or even water soluble substrates such as glucose. Surface tension measurements confirmed biosurfactant production by the isolated bacteria. Glucose-grown cultures of the I-15 isolate reduced the surface tension of the growth medium from 68 mN/m to ca 40 mN/m (ca 40 % reduction). Whereas crude oil-grown cultures of the I-19 strain brought about 20% reduction in surface tension as compared to that of the uninoculated medium. The CMC of the biosurfactant recovered from cultures of I-15 strain on glucose was estimated to 200 mg/L. The biosurfactant caused a reversible inhibition of the growth of the I-15 strain on glucose. Fourier Transform Infrared Spectroscopy of the biosurfactant recovered from the I-15 cultures on glucose revealed functional groups that are typical of a lipopetide

biosurfactant. The putative lipopeptide has potential applications in the petroleum industry and environmental bioremediation. Moreover, it could be used as an antimicrobial agent.

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3- Krishnaswamy, M., Subbuchettiar, G., Thiengungal, K. R., and Panchaksharam, S.(2008). Biosurfactants: Properties, Commercial Production and Application. Current Science. Rev.VOL. 94.

OTP140

A diagnostic qPCR assay for detection and quantification of emetic and non-emetic *Bacillus cereus* in milk

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Question: *Bacillus cereus* is known as the causative agent of an emetic and a diarrheal type of food-borne illness, and thus is a special problem for public health issues and for the dairy industry. Therefore more precise monitoring of *B. cereus* is necessary for a better understanding of their contribution to health and disease.
Methods: The aim of the present study was to develop a diagnostic real-time quantitative PCR (qPCR) for the *B. cereus* group in milk. A TaqMan qPCR assay based on amplification of the gyrase B (*gyrB*), the *ces* emetic toxin and *the 16S rRNA* target sequences was designed including an internal amplification control (IAC) to identify false negative results.
Results: The method showed 100% inclusivity and exclusivity when testing a panel of 41 *B. cereus* group strains, 10 non-*B. cereus* group strains and 17 non-bacilli strains. The IAC target included in each qPCR reaction showed no interference with the main reaction. The detection limit was successfully established in artificially contaminated raw milk samples and the optimized assay applied to naturally milk contaminated samples.
Conclusions: The qPCR assay is specific and sensitive and provides an efficient diagnostic and monitoring tool for the identification of the *B. cereus* group in food.

OTP141

ROS formation by photochemical reactions affect BCC in a humic lake and induce adaptive responses in abundant bacteria

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Sunlight-mediated photochemical reactions of colored dissolved organic matter (CDOM) is an important process in humic lakes enhancing substrate availability for heterotrophic bacterioplankton. Although bacterioplankton species benefit from generated carbon substrates they have to cope with toxic reactive oxygen species (ROS) generated simultaneously. We investigated effects of artificially increased singlet oxygen ($^1O_2$) formation and hydrogen peroxide (H_2O_2) concentrations on bacterioplankton community composition (BCC) in the subsurface water layer of the humic Lake Grosse Fuchskuhle.
BCC changes of abundant and metabolically active bacteria were investigated by the generation of 16S rRNA gene clone libraries and 16S rRNA targeting RT-PCR DGGE analysis using *Bacteria* and group-specific primer-systems.
Major bacterioplankton groups respond differently to $^1O_2$ and H_2O_2 exposure. *Alphaproteobacteria* (*Novosphingobium acidiphilum*) and *Betaproteobacteria* (*Polynucleobacter necessarius* and *Limnohabitans* related species) increased in relative abundance after $^1O_2$ but not after H_2O_2 exposure. In contrast freshwater *Actinobacteria* were not detected after $^1O_2$ exposure but increased in relative abundance after H_2O_2 exposure. We were able to isolate strains representing the above-mentioned *Alpha-* and *Betaproteobacteria* and used those for laboratory and in situ studies to investigate the response to ROS exposure. First experiments showed that those strains were capable to withstand increased $^1O_2$ exposure after pre-incubation with moderate $^1O_2$ concentrations occurring regularlyin the investigated ecosystem. Our results indicate that ROS generation by CDOM photolysis is an important factor for BCC in humic lakes and favor species with adaptive response mechanisms to ROS exposure.

Glaeser SP., Grossart, H.-P. , and J. Glaeser (2010) Environ Microbiol 12(12): 3124-36

OTP142

Gluconobacter oxydans as a platform for the production of industrially important products

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Many useful organic compounds, such as pharmaceuticals and food additives, contain asymmetric carbon centers and enantionmeric forms exist. Chemical synthesis of these products is often troublesome and produces racemates. It is common to have a single biologically active enantiomer, while the other does not show activity and sometimes has a harmful effect. In such cases chemically synthesized racemates usually need to be resolved, especially for pharmaceuticals. In contrast, many enzymes act regio- and stereoselectively and are naturally capable of converting pro-chiral educts to enantiopure products. *Gluconobacter oxydans* is an important organism in biotransformation (e.g used in vinegar, vitamin C and antidiabetic drug production). Its genome is known[1] and contains many uncharacterized cytosolic and membrane-bound dehydrogenases/oxidoreductases (>70) and they were surveyed for their ability to produce industrially important chiral products. Investigation into protein function via heterologous gene production in *E. coli* revealed many oxidoreductases that reduced α,β-diketones, α-ketoaldehydes, and vinyl ketones. These enzymes are capable of producing chiral building blocks that find uses in industry (e.g. pharmaceutical, food additives and fragrance). Four cytoplasmic oxidoreductases were capable for producing hydroxy carbonyls with chiral centers[2]. Additionally, three cytoplasmic reductases acted on the olefinic bonds of vinyl ketones, two of which produced stereospecific products when the olefinic bond was substituted[3]. A cofactor regeneration scheme was developed to decrease costs and increase yields. Membrane-bound dehydrogenases do not need cofactor regeneration and those of *G. oxydans* are known to excrete their incomplete oxidation products of sugars, polyols, and alcohols to almost quantitative yields into the medium. Accordingly, the numerous membrane-bound dehydrogenases of *G. oxydans* were found to oxidize an array of diols and polyols, likely to chiral hydroxy carbonyls. Identification of the enzymatic products is currently ongoing. Consequently, *G. oxydans* enzymes are renewable resources that provide a platform for the production of optically active products in high amounts and avoid the toxicity often involved in multi-step organic synthesis.

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OTP143

Microbial quality of table eggs sold in some Libyan market

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High development in commercial poultry rearing in Libya play an important role in the creation of income and also provide food in form of meats and eggs , in Libya consumption of eggs per person per week about six eggs.
The eggs considered to be highly nutritional value containing high levels of vitamins and minerals, although the eggs considered a source of complete food for growth but there are a lot of researches indicate that micro organisms often contaminate eggs.
Total of 150 samples were collect randomly from different Libyan markets in Tripoli area and area surround Tripoli.
Total count of bacteria and fungi were performed to all samples.
The result showed that there were high levels of bacteria Isolated from eggs content in different percents,E.coliwas more frequency and pseudomonas spp were highly frequent and Aeromonas .spp

OTP144

Simple and Rapid Detection Of Salmonella spp from Cattle farms using Polymerase Chain Reaction in Arak, Iran

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This study goal to employ biochemical and molecular assays to detect and diagnose
Salmonellain cattle farms in Markazi province in central part of Iran. For this reasone,1124 faecal samples were collected from cattle randomly. Selective culture media specific for Salmonella were used to grow a number of colonies from cattle samples. Salmonella suspicious colonies were confirmed using biochemical tests. After biochemical confirmation, the isolates were subjected to molecular based approach to identify

Salmonella spp by amplifying specific genes 16s rDNA gene. The PCR products were analyzed on 1% agarose gel. Thirty six samples were found as a positive among of 1124 collected samples. The dada shown that molecular based approaches are more rapid and should thus be used for any initial detection of Salmonella SPP.

OTP145

Systematical approach to decipher the rationales behind the detergent and solvent stability of a model lipase

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The stability and activity of the biocatalysts is often compromised by the use of certain additives, e.g. detergents and organic solvents, to increase the solubility of certain reactants.
This effect is not surprising and due to the evolutionary design of the enzyme. Enzymes have been evolved by nature to work efficiently in aqueous environments and thus require a water shell surrounding the protein surface to retain enzymatic activity. Solvents and detergents interfere with the surrounding water shell and protein electrostatics. This interference can lead to the unfolding and aggregation and a loss of activity. Despite these effects the influence of solvents on the enzyme structure and function has neither been studied systematically nor been understood theoretically so far.
We aim to discover the potential of stabilizing a model enzyme in non-conventional media through a systematic mutagenesis study. We are interested in the development of a predictive stability model for the customized design of biocatalysts in respect to the intended application. The model enzyme for our purpose is BSLA (LipA from Bacillus subtilis), a minimal α/β-hydrolase which can be easily expressed in Escherichia coli. BSLA has already been well characterized and is of known structure, the biotechnological potential has been demonstrated with the production of enantiopure cyclohexane-trans-1,2-diol[1].
In preparation of this screening we have performed a saturation mutagenesis along the whole sequence of BSLA. Degenerated codons were used to substitute the wild type amino acid by every other naturally occurring amino acid, resulting in a total of 3439 BSLA variants (181 amino acids x 19 possible substitutions). We are now developing a high throughput screening system to monitor the stability of every variant in different detergents and organic solvents. The selection of the solvents is justified through different interferences towards the intra protein interactions that will be weakened. The results will give us an insight into the contribution of every single amino acid towards the stability of the whole enzyme. The library construction and mutant screening is performed in cooperation with a project partner(b) which will focus on the stability in other non-conventional media. We will present the results from the screening of several exemplary mutants.

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OTP146

Evaluating Food Safety Management Performance in a Milk Pasteurising Facility using a Microbiological Assessment Scheme

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Milk and milk products are a heterogeneous group of food products. Depending on the heat treatment applied during production, different pathogens pose risks. The pathogens of concern are *Listeria monocytogenes*, *Bacillus cereus*, *Salmonella* spp, *Staphylococcus aureus and Escherichia coli since* these may survive pasteurisation treatments. The performance of a food safety management system (FSMS) in a drinking milk pasteurisation establishment was measured using a microbiological assessment scheme (MAS). The MAS consisted of multiple sampling locations along the processing line consisting of high-risk raw materials, the processing environment, process water and end products. A total of 1268 samples were analysed over an 18-month-period. Nine microbial parameters (*Salmonella* spp., *Listeria spp., B cereus, Staph. aureus*, Total Bacterial Counts (TBC), Enterobacteriaceae, *E. coli*, Faecal *enterococci* and coliforms) were assessed. Results were benchmarked against legal, industry and best practice norms. 100% (n_0 = 233) of raw milk samples met the EU TBC standard of < 10^5cfuml^{-1}, however, *Listeria innocua* was isolated in 3% (n_1=134) of raw milk samples. *Listeria* spp. (n_2=128), *Salmonella* spp. (n_3=118), *Staph. aureus* (n_4=118), Enterobacteriaceae (n_5=114), *B. cereus* (n_6=38) and *E. coli* (n_7=23) were not detected in any end products. *Listeria welshimeri* (a poor hygiene indicator) was identified in 2% (n_8=153) of environmental samples Salmonella was not isolated in 63 environmental sample. 6% and 1% of operator hand swabs (n_9=100) had TBC and Enterobacteriaceae counts respectively in excess of best practice norms of 10^2cm^{-1} and 10^1cm^{-1} respectively. One (2.2%) water sample (n_{11}=46) had a coliform count of 201cfuml^{-1} whereas five samples (11%) had TBC counts above acceptable norms. The results indicate that the FSMS is producing a safe product. The MAS is an effective risk assessment tool that is useful to assess the overall performance of the FSMS and allows a more targeted use of resources to implement improvement. Satisfactory end product microbiological results indicate that cold chain control, post pasteurisation contamination from dry ingredients (e.g. buttermilk cultures), packaging or unsanitary pipe work are not issues for this plant. However, the prerequisites of environmental sanitation, raw material supply and control, water treatment and storage and staff hygiene are the areas within the FSMS that pose the greatest risks.

OTP147

Salmonella contamination of a Category 3 fat rendering plant - a case study

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Safe petfood production is a key objective of manufacturers. Petfoods and treats are often found in the home food preparation areas. Petfoods are often handled by children and the elderly. Food safety issues involving direct human contact with processed petfoods is becoming a major regulatory focus. This case study describes an intractable case ofSalmonellacontamination in a Category 3 animal by-products rendering facility that produces tallow for the oleo-chemical industry and greaves for petfood manufacture. The facility is located adjacent to a beef slaughterhouse operates a Hazard Analysis and Critical Control Point (HACCP) based manufacturing system. The HACCP plan identifies three Critical Control Points (CCPs) - pre-rendering particle size, metal detection and rendering temperature and duration. The facility is approved under Regulation (EC) 1774/2002 and is subject to official controls by the Competent Authority. Over a period of 5 years 33 of 305 official greaves samples intermittently revealed the presence of *Salmonella anatum*, *S. kentucky* and *S. newington*. No deficiencies were detected in CCP implementation. Due to the high rendering temperatures the source of contamination was believed to be post rendering contamination.Salmonellawas not isolated from any of the environmental samples (n = 62) nor from the products taken within process (n= 88). Analysis of pre-requisites identified deficiencies in pest control, sanitation, zoning, operator hygienic practices and structure fabrication. Deep cleaning and corrections to operational pre-requisite resulted in temporary improvements. The establishment was decommissioned for 10 months. Prior to re-opening fabrication was improved by laying a smooth floor, removing roughened welded seams in equipment, smooth plastering the walls and properly ducting cables and hoses. Post structural improvement, none of the 120 official greaves samples revealed the presence ofSalmonella. The likely contamination source is from intermittent shedding from nidi located in the deep recesses of blemishes within the fabric.Salmonellais capable of surviving for extended periods in a variety of environments. Complete elimination of pathogens is dependent on the strict adherence to HACCP and GMPs. However, some practices are easy to apply, however in this case restoration of control required significant investment and plant redesign.

OTP148

Salmonella as a process hygiene microbiological criterion in Irish Wild Pheasant

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Microbiological criteria provide guidance on the acceptability of foodstuffs and their HACCP-based manufacturing processes. Regulation (EC) 2073/2005 establishes process hygiene criteria (PHC) for carcasses of domestic fowl. No such criteria exist for pheasant. It is thus appropriate to establish PHC. The processor selected for the study procures pheasants hunted from protected reserves, which are stocked with 18-week-old pullets from a rearing unit 3-4 months prior to the shooting season. In season 1 on each of 10 processing days 4g of the neck skin (NS) were aseptically harvested from 35 pheasants selected at random post-chilling. The NS from 7 carcasses were pooled to create 5 x 25g final samples. Samples were analysed for the presence forSalmonellausing ISO method 6579. One sample revealed the presence of *Salmonella*. This procedure was repeated in seasons 2 and 3 with identical results. PHC for pheasant were determined thus:n= number of units compromising the sample = 50 derived from 10 consecutive sessions;c= number of samples where *Salmonella* is detected = 1;m = M= absence in 25g of a pooled NS sample. Ongoing performance exceeding these criteria prompts the establishment to implement timely corrective action to its processing procedures and to review disease control and bio-security measures on the rearing farm. In

the absence of legally mandated PHC for pheasant, it is recommended that processors follow the protocols outlined to establish their own PHC.

OTP149

Effects of drought and rewetting on bacterial community structure and extracellular enzyme activity in stream-bed sediments

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Increasing temperatures caused by global climate change affect stream ecosystems as more frequent and longer drought events and more frequent and severe floods occur. In the current study bacterial community structure (CARD-FISH) and the activity of five extracellular enzymes have been investigated after artificial drought and rewetting of stream sediments. Sediment from the Breitenbach (Hesse) was dried at 20°C over 13 weeks in plastic boxes with different covers simulating fast, intermediate, and slow drying before rewetting with untreated or sterilized stream water using the perfused core technique for 14 days. The total number (SybrGreen) of prokaryotes decreased after 2 weeks at all treatments. The community in the initial wet sediment was dominated by Betaproteobacteria and shifted during drying to a dominance of Alphaproteobacteria and Actinobacteria in all treatments. After only 1 day of rewetting in the treatment with untreated stream water and after 2 days with sterile stream water the Betaproteobacteria recovered. The activity of the enzymes was affected by drought but did not vanish. Potential aminopeptidase and alpha-glucosidase activity in the fast and intermediate drought treatment decreased distinctly within 4 weeks whereas phosphatase, beta-glucosidase and -xylosidase activity decreased less severe. In the rewetting experiment the activity of alpha-glucosidase and aminopeptidase was increasing fastest but activities of all enzymes remained below the initial values after 14 days. The results demonstrate that the microbial community in stream-bed sediment was highly affected by drought but recovered fast when rewetted. Drought facilitated Actinobacteria and Alphaproteobacteria. In the view of ecosystem function the degradation of proteins was affected first. All tested enzymes did not disappear completely after 13 weeks of drought.

OTP150

Antimicrobial effects of silver nanoparticles against microorganisms from activated sludge

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The extensive use of engineered metal nanoparticles with antimicrobial properties (*e.g.*, Ag, Zn, Cu, Ce, Ni) and their increased release into the environment has raised major concerns, due to unexplored (eco)toxicological effects and inadequate testing methods.
Samples were taken from activated sludge of a municipal wastewater treatment plant (Giessen, Germany) and suspensions in aqueous buffer solutions were spiked with commercially available Ag(0) nanoparticles. Size distribution of the suspended Ag(0) nanoparticles was determined by Nanoparticle Tracking Analysis and revealed an average particle size of 31 nm (D90 <45 nm). Adverse effects on microorganisms were monitored using an automated assay in 96-well microtiter plates, which allowed simultaneous cultivation and real-time analysis of nanoparticle-mediated growth inhibition. Concentrations above 0.01% (w/v) revealed complete and irreversible growth repression. Ag(0) concentrations of ≥0.002% resulted in partial inhibition, correlated with extended lag-phases and reduced optical density in the plateau phase. In contrast, the maximum slope of Gompertz functions fitted to microbial growth curves was not an indicator for inhibition by Ag(0) nanoparticles. DGGE fingerprinting of amplified 16S rDNA fragments was used to follow the temporal dynamics of the community structure in the presence of different Ag(0) nanoparticle concentrations. DGGE patterns were clearly different in the range from 0.005% to 0.01% Ag(0).
Hydrolytic enzyme activities involved in the cycling of carbon, nitrogen, phosphor and sulfur were analyzed using 4-methylumbelliferyl (MUF)-conjugated model compounds and kinetic fluorescence measurements. Compared to controls without silver treatment, reduced activities with MUF-conjugated substrates could be measured for α-glucosidase, β-glucosidase, N-acetyl-glucosaminidase, sulphatase and phosphomonoesterase, in part at silver concentrations of ≤0.005%. Tests in mineral media demonstrated Ag(0) growth inhibition at much lower and environmentally relevant silver concentrations, most likely due to sorption of Ag(0) to constituents in the complex media and interference by Ag(0) nanoparticle reaggregation.
Testing the metal toxicity in experiments with bulk silver (Ag(0) flakes, particle size 10 μm) also resulted in marked growth inhibition, indicating that merely a portion of the observed susceptibility effects can be attributed to nanoscale-based silver toxicity.

OTP151

Influence of the microbial community and mineral formation on the biogas production process

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In order to reduce the effects of climate change due to increasing CO_2 emissions, it is necessary to develop technologies to use renewable resources more efficiently.
One solution is the anaerobic fermentation of organic waste material. Due to the lack of understanding, biogas plants are often run under their maximum loading rate. To optimise the plant operation, it is important to gain a better understanding of microbial biocenosis and its behaviour under stress conditions, e.g. increasing organic loading rate. In order to maximise the space-time-yield in a biogas plant, we need to investigate the changes of the microbial community during shock loads, over-acidification and deacidification.
In the present study we have monitored the microbial biocenosis during the changing of process conditions within several laboratory experiments to examine the effects of over-acidification. The biological samples were examined using different molecular biology methods, to observe changes in the abundance and activity of the microorganisms involved. In addition to the characterisation of the dominant species of the microbial community with genetic fingerprinting (*Denaturing gradient gel electrophoresis, DGGE), we used*qPCR (quantitative real-time-polymerase chain reaction) and FISH (*fluorescence*in situ hybridization) for the quantification of different groups of microorganisms and their metabolic activity.
During organic overloads the influence of deacidification with different bases on the biocenosis was determined. Furthermore, we have performed microbial monitoring during increasing of the space-time-yield. We increased the organic loading rate four-fold about the normal level. Nevertheless the process of biogas formation runs stable all the time.
While these experiments the formation of different aggregates was observed, that had a positive influence on the process stability. The size of aggregates was depended on the amount of additives used to stabilize the process during over-acidification.
Additives have a positive influence on the biogas production process. Investigations of the microbial community influenced by the addition will bring new insights into the stability of the biogas process.

OTP152

A naphthopyrone synthase-like PKS from *Aspergillus terreus* produces phytotoxins

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Aspergillus terreus is a saprophytic filamentous fungus with its natural habitat in soil, compost or associated with decaying fruit. *A. terreus* has a large potential to produce a wide variety of different secondary metabolites. However, it lacks a polyketide synthase (PKS) gene conserved in all related *Aspergillus* species that produces a naphthopyrone derivative responsible for colouration of conidia. Here, we discovered that in *A. terreus* the PKS most closely related to naphthopyrone synthases produces a phytotoxin. Analysis of HPLC profiles from a PKS deletion mutant revealed that it is required for the synthesis of at least 15 different metabolites, among them the major metabolite terrein. This well-known phytotoxin is a strong antioxidant that shows weak toxicity to mammalian cells but potently harms the surface of several fruits. Using a beta-galactosidase reporter strain we observed a weak expression of the gene cluster on minimal media and moderate activation on complex media. Interestingly, expression strongly increased in presence of plant derived compounds such as malt extract or different fruit juices. This indicates a specific recognition of yet unknown plant compounds resulting in phytotoxin production. Further analyses of the gene cluster and its metabolites are currently under investigation. Additionally, the potential of metabolites in inhibiting root growth of plants is addressed.

OTP153

Transcriptional regulation of the operon encoding the stress-responsive sigma factor SigH and its anti-sigma factor RshA, and control of SigH regulatory network in *Corynebacterium glutamicum*

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Expression of genes in *Corynebacterium glutamicum*, a Gram-positive non-pathogenic bacterium used mainly for industrial production of amino acids, is regulated by seven different sigma factors of RNA polymerase, including stress-responsive SigH. According to the *C. glutamicum* genome sequence, the SigH-dependent transcription may be controlled by anti-sigma factor encoded by the *rshA* gene. The aim of the study was to analyze transcriptional regulation of thesigHandrshAgenes, prove the function of *rshA*, determine the genes of the SigH regulon and propose a model describing the role of SigH and RshA in oxidative and heat stress responses.
Transcription analysis revealed that the *sigH* gene and anti-sigma *rshA* gene are cotranscribed from four *sigH* housekeeping promoters in *C. glutamicum*. In addition, a SigH-controlled *rshA* promoter was found to drive separate transcription of the *rshA* gene. To test if transcription of SigH-controlled genes is increased in the absence of the anti-sigma factor *rshA* gene under standard growth conditions, a *C. glutamicumr shA* deletion strain was constructed and used for genome-wide transcription profiling. In total, 83 genes organized in 61 putative transcriptional units, including those which were previously detected using *sigH* deletion strains, exhibited increased transcription in the *rshA* deletion mutant in comparison to the wildtype strain. The genes encoding proteins related to disulphide stress response, heat stress proteins, components of the SOS-response to DNA damage and proteasome components were the most apparent upregulated gene groups. Potential SigH-dependent promoters upstream of the identified genes were found by transcription start determination and by sequence analysis.
The *rshA* gene codes for an anti-sigma factor controlling function of the stress-responsive sigma factor SigH in *C. glutamicum*. Transcription of *rshA* from a SigH-dependent promoter may serve to quickly shutdown the SigH-dependent stress response after the cells have passed the stress condition. We propose here a model of regulation of oxidative and heat stress response including the redox homeostasis by SigH, RshA and thioredioxin system. The updated consensus sequence of SigH-controlled promoters was derived from the 45 promoters of the genes belonging to the SigH regulon.

OTP154

Nanoflagellate diversity during the iron fertilization experiment LOHAFEX

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According to the iron hypothesis of J. Martin, vast parts of the ocean are nutrient rich but iron limited. Therefore, fertilization of these areas with iron sulfate, in order to create algae blooms, was considered as a method of CO_2 sequestration. The main aim of the study was the investigation of side-effects of such events to the ecosystem. Since investigations of the bacterial community discovered a strongly top down controlled system, investigation of the next larger organism, the eukaryotic plankton < 20μm, may help to shed light into the black box of biological carbon pump. The <20 μm fraction of the plankton consists mainly of nanoflagellates. This group of organisms is taxonomically highly diverse and only little is known about their role in marine ecosystems. Within the nanoflagellates autotrophic, mixotrophic and heterotrophic species can be found. Autotrophic nanoflagellates play a role as primary producer in marine systems, whereas heterotrophic nanoflagellates (HNF) graze upon *Bacteria* and *Archaea* of a size range between 1 μm and 3 μm. Mixotrophic organisms are phototrophic and ingest bacterial prey at the same time. Thus heterotrophic and mixotrophic organisms develop a top down control on the microbial community, especially on large bacteria such as *Gammaproteobacteria* or *Roseobacter*. During LOHAFEX a phytoplankton bloom consisting mainly of the *Prymnesiophytaceae Phaeocystis* spec. was induced and monitored over 38 days. To investigate this nanoflagellate community within this bloom we used catalyzed reporter deposition fluorescence in situ hybridization (CARD FISH) and automated cell counting, flow cytometry and 454 tag pyrosequencing. The nanoflagellate community consisted mainly of *Prymnesiophytes*, *Prasinophytes* and *Syndiniales* clades, while marine *Straminopiles* played a minor role. Beside the mixotrophic *Phaeocystis* a stable community of HNF was found within the bloom. This HNF community was found to apply a strong top down control on the microbial community during LOHAFEX and thus playing a major role in the microbial loop and the efficiency of the biological carbon pump. Here we report the changes of the nanoflagellate community during the 38 days of the iron fertilization experiment LOHAFEX.

OTP155

Microbial diversity along the gut system of the Desert Locust (*Schistocerca gregaria*) as revealed by 16S rRNA pyrotag sequencing

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Swarms of the desert locust *Schistocerca gregaria* can cause devastation of huge areas. Former work suggests high efficiency in lignocellulose degradation backed by the gut microbiota. This study aims to elucidate the microbial diversity along the locust's alimentary system. Samples from different gut sections (i.e. salivary glands, foregut, midgut, gastric caeca, hindgut) of laboratory-bred and wheat-fed gregarious adults were analyzed via 454 pyrotag sequencing. Raw sequencing data were analyzed using both the pyrotagger and the Qiime software tools. The highest diversity was found in the foregut while the composition in the other parts is relatively simple. In some samples a high fraction of reads (up to 50%) belonged to previously not recognized OTUs (i.e. 16S rRNA sequence identity <90% to any sequence in public databases). The most abundant phyla are Proteobacteria (mostly Enterobacteria) and Firmicutes (mostly Lactobacillales). Furthermore eukaryotic gut symbionts (e.g. Ascomycota, Ciliates) were found. Since the information content of the pyrotag data with approx. 200 nt is not sufficient for reliable phylogenetic analysis we set out to prepare clone libraries of larger parts of the small subunit ribosomal RNA, 16S and 18S, respectively. Additionally, unknown gut microorganisms are isolated and cultivated for further characterization.

OTP156

Characteristics of the main bacterial strains causing potato complex scab diseases in Hamedan province (Iran)

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Several *Streptomyces* species are reported from potato growing area in the world which cause potato scab diseases. Main pathogenicity factor among these species are *nec1* and thaxtomine biosynthesis genes including *txtA, txtB, txtC* and *txtD*. As potato scab disease is an important disease in Hamedan province, samples which shown scab symptoms including raised, netted and deep or shallow-pitted lesions collected from the main potato fields. The causative agent *Streptomyces* strains were isolated and based on the phenotypic features and induced symptoms they were very heterogeneous and were belong to four main groups: *S. scbies, S. acidiscabies, S. turgidiscabies* and *Streptomyces* sp. They were pathogenic on potato, parsnip, horse radish, carrot and some other tested plants. Raised and netted potato scab-inducing strains produced thaxtomin determined by thin layer chromatography, but this phytotoxin could not be detected in the pitted lesion-inducing strains. Selected strains were examined for the presence and situation of the pathogenicity related genes as they induced variable disease symptoms under field and green house condition. Pulsed field gel electrophoresis technique revealed that most of the tested strains carried a linear plasmid. Amplification of the pathogenicity genes fragments and southern hybridization analysis showed that only some tested trains harbornec1andtxtgenes. A total of 20 representatives strains were grown on modified oatmeal medium and extracted with ethyl acetate. It was shown that some strains produced pathogenicity compound(s) other than thaxtomin which induced pitted lesion on potato tuber slice. Searching for the presence new pathogenicity factor and pathogenicity related gene(s) among the representative of the deep pitted potato scab inducing strains is under investigation.

OTP157

The role of Methanotrophy in carbon turnover in the Lena Delta

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Great amounts of methane are stored in permafrost areas in the Delta of the Lena River, the north-eastern part of Siberia. Due to predicted increasing temperatures, permafrost surrounding the Lena will melt at increasing rates which can result in increased input of carbon, either as methane or as other organic compounds. Methane concentrations and oxidation rates were measured in lakes and streams of Samoylovskiy Island and in the surrounding Lena River. Highest methane concentrations (up to 1000 nM) were found in very shallow streams connecting the lakes with the river,

where lowest concentrations were recorded. Methane oxidation rates mostly followed this pattern. Experiments simulating the mixing of freshwater methanotrophic bacteria from the river with the saline waters of the Laptev Sea indicate that at salinities above 5 PSU their function as biofilter ends.

OTP158
Inhibition of the anaerobic degradation of ethylene glycol by benzotriazoles

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1H-benzotriazole and its methylated derivative tolyltriazole belong to the most frequently used corrosion inhibitors in borehole heat exchanger systems.In case of a leakage, a local groundwater contamination might occur where ethylene glycol-based heat transfer fluid containing corrosion inhibitors enter the aquifer down to a depth of 150 meters. Microcosm experiments with sediment inoculum showed that the two corrosion inhibitors are resistant to biodegradation under sulfate-, nitrate- and iron-reducing conditions. This study describes the inhibitory effect of benzotriazoles on ethylene glycol degradation under nitrate- and sulfate-reducing conditions.
Experiments were conducted using a sediment inoculum from a depth of 60 meters, which was sampled during the installation of a borehole heat exchanger system. The biodegradation of ethylene glycol (5 mM) was assessed as the sole carbon source and in the presence of (50 μM) of each of the benzotriazoles. Microcosm experiments were performed in triplicate at 12°C and room temperature (RT).
In the absence of benzotriazoles more than 98 % of the initial ethylene glycol was degraded within eight days by the denitrifying bacteria. In the presence of the two corrosion inhibitors the degradation of ethylene glycol proceeded at a lower rate and 98 % of the substrate were not degraded until 15 days of incubation. Under sulfate-reducing conditions 50-100% of the initial ethylene glycol concentration was utilized within 138 days of incubation in the absence of benzotriazoles. The presence of 1H-Benzotriazole caused inhibition of the biodegradation of ethylene glycol at lower temperatures. In the presence of tolyltriazole the effect on the ethylene glycol degradation was variable, which might be explained by the heterogeneous distribution of microorganisms in the inoculum.
These findings indicate that benzotriazoles may not only threaten groundwater quality due to their own toxicities but in addition inhibit the biodegradation of other organic compounds.

PSV001
The unusual cell architecture of *I. hospitalis* and consequences for its energy metabolism

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The members of the genus *Ignicoccus* belong to the phylum of the Crenarchaeota. They obtain energy chemolithoautotrophically by the reduction of elemental sulfur with molecular hydrogen as electron donor (1). All described *Ignicoccus* species exhibit a unique cell architecture that differs from all other Archaea known so far. The cell envelope consists of two membranes enclosing a huge inter-membrane compartment (IMC). Surprisingly, it was shown for *I. hospitalis* that the outermost membrane contains the H_2:sulphur oxidoreductase as well as the ATP synthase. Thus, *I. hospitalis* is the first organism with an energized outermost membrane and ATP synthesis within the IMC. DAPI staining and EM analyses showed that DNA and ribosomes are localized in the cytoplasm, leading to the conclusion that energy conservation is separated from information processing and protein biosynthesis (2). In addition, we were able to demonstrate that the acetyl-CoA synthetase that activates acetate to acetyl-CoA is associated to the outermost membrane. This is the first energy-consuming process proven to take place in the inter-membrane compartment.
To further investigate the energy metabolism under these extraordinary conditions, we are working on the purification and characterization of the complete ATP synthase complex of *I. hospitalis*. This includes studies on the stability of the enzyme complex, its molecular composition, and its behaviour against inhibitors. The findings of these experiments also will shed light on the nature of the intimate association between *I. hospitalis* and *Nanoarchaeum equitans* (3). It is known that *N. equitans* receives amino acids and lipids from its host. At present, it is still unclear if the energy metabolism of *N. equitans* is dependent on *I. hospitalis*, too. Finally, a re-examination of the nomenclature of the different compartments and the two membranes of *I. hospitalis* will be discussed.

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(4) This project is supported by a grant from the DFG

PSV002
Function and specificity of the dual flagellar sytem in *Shewanella putrefaciens* CN-32

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Bacteria move towards favorable conditions by rotating helical proteinaceous filaments, called flagella. The motor part of this intricate bacterial nanomachine incorporates stator units that exert torque on the filament using gradients of H^+- or Na^+-ions. Stator units and the rotor component FliM can be dynamically exchanged during function. Previous studies have shown that a large number of microorganisms harbor dual flagellar systems. However, little is known about function and regulation of dual flagellar systems in many species.
The γ-proteobacterium *Shewanella putrefaciens* CN-32 possesses a complete secondary flagellar system along with a corresponding stator unit. In contrast to most secondary flagellar systems that have been studied so far, expression already occurs during planktonic growth in complex media and leads to the formation of a subpopulation with one or more additional flagella at random positions in addition to the primary polar system. We used physiological and phenotypic characterizations of defined mutants in concert with fluorescent microscopy on labeled components of the two different systems, the stator proteins PomB and MotB, the rotor components $FliM_1$ and $FliM_2$,and the auxiliary motor components MotX and MotY, to determine localization and function of the proteins in the flagellar motors.
Our results demonstrate that the polar flagellum is driven by a Na^+-dependent $FliM_1$/PomAB/MotX/MotY flagellar motor, while the secondary motor is rotated by a H^+-dependent $FliM_2$/MotAB motor. There is strong evidence that these components are highly specific for their corresponding motor and are unlikely to be extensively swapped or shared between the two flagellar systems under planktonic conditions. The results have implications for the specificity and dynamics of flagellar motor components.

PSV003
Pyruvate formate-lyase Controls Formate Translocation by the FocA Channel

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Formate is one of the major products of mixed-acid fermentation in Enterobacteria such as *Escherichia coli* and is an important electron donor for many anaerobes. During fermentation in *E. coli* up to one third of the carbon derived from glucose is metabolized to formate. The final step is catalyzed by the cytoplasmic enzyme pyruvate formate-lyase (PflB), which catalyses the homolytic cleavage of pyruvate to acetyl-CoA and formate. PflB is a glycyl-radical enzyme that is converted from an inactive to an active form by the radical-SAM enzyme PflA[1].
Because accumulation of formate inside the cell can lead to acidification of the cytoplasm a mechanism to regulate its intracellular level must exist. FocA is a bidirectional formate channel protein that belongs to the family of formate-nitrite transporters (FNT)[2]. Its gene, *focA*, is co-transcribed with that encoding PflB. Although several structures of FocA have been published recently[3], there is still no clear mechanistic understanding of how formate import and export by FocA is controlled. Because synthesis of FocA and PflB is highly coordinated this suggested that PflB might play a key role in controlling formate translocation across the cytoplasmic membrane. In initial experiments we could show a FocA-dependent interaction of PflB with the cytoplasmic membrane. The specificity of the FocA-PflB interaction could be subsequently confirmed using a variety of in vivo and in vitro experimental approaches. Our findings indicate that it is the inactive form of PflB that interacts with FocA. Based on these findings we developed an assay to test our model for PflB-controlled gating of formate transport by FocA in vivo.

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PSV004

The small non-coding csRNAs controlled by the response regulator CiaR affect ß-lactam sensitivity and competence in *Streptococcus pneumoniae*

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The two-component regulatory system CiaRH of *Streptococcus pneumoniae* directly controls 15 promoters, which drive transcription of 24 genes organized in 5 operons and 10 single transcriptional units. Five of these monocistronic units specify small non-coding RNAs, designated csRNAs (cia-dependentsmall RNA) (1). Expression analyses of the CiaR regulon demonstrated that CiaRH maintains high levels of gene expression rather than responding strongly to a signal (2). Hyperactivation of the regulon by mutations in the histidine kinase gene *ciaH* leads to increased ß-lactam resistance and concomitantly to a block of genetic competence (3). To determine which constituents of the CiaR regulon are involved in these phenotypes, gene inactivation studies were performed in strains with an activated CiaRH system. The results of these experiments showed that the block of transformability as well as the increased ß-lactam resistance is mediated by the csRNAs. Testing csRNAs individually revealed a dominant role for csRNA4 and csRNA5 in both phenotypes. Genomic searches for complementarity to csRNAs yielded no apparent candidates for ß-lactam resistance, but *comC* for competence regulation. The gene is coding for the precursor of the secreted competence stimulating peptide CSP, which is needed to initiate competence development in *S. pneumoniae*. Analysis of *comC* translational fusions in the presence or absence of csRNAs demonstrated post-transcriptional control of *comC* expression. In addition, partial disruption of *comC*-csRNA complementarity by mutagenesis relieved *comC* from csRNA-mediated control. Thus, the CiaRH system interferes with quorum-sensing regulated competence development via small non-coding csRNAs.

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PSV005

Fur mediates control of riboflavin biosynthesis, iron uptake and energy metabolism in *Clostridium acetobutylicum*

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A system for maintenance of adequate iron status within the cell in most bacterial species is represented by Fur (ferric uptake regulator). The role of this regulator in the bacterial iron response has been an area of active investigation. However, the molecular mechanisms for maintenance of iron homeostasis in strictly anaerobic bacteria have remained largely uncharacterized. *C. acetobutylicum* is a representative of this group. A unique feature of its fermentative metabolism is the ability to switch from synthesis of the organic acids acetate and butyrate during exponential growth to production of the solvents butanol, acetone and ethanol upon transition to stationary phase. A gene coding for a putative ferric uptake regulator has been identified in the genome of *C. acetobutylicum*. We inactivated the *fur* gene using insertional mutagenesis. The resultant mutant showed a slow growing phenotype, but essentially no drastic change in its fermentation pattern. A unique feature of its physiology was the overflowing production of riboflavin. To gain further insights into the role of the Fur protein and the mechanisms for establishment of iron balance in *C. acetobutylicum*, we characterized and compared the gene expression profile of the *fur* mutant and the iron limitation stimulon of the parental strain. In accordance with the phenotypic profile of the mutant, the genes that compose the *rib*DBAH operon, involved in riboflavin biosynthesis, were highly upregulated. Proteomic analysis of the fur mutant further confirmed these results. The rib genes were also highly induced, when the wild type strain was challenged with conditions of iron limitation. Not surprisingly, a repertoire of iron transport systems was upregulated in both microarray datasets, suggesting that they are regulated by Fur according to the availability of iron. Furthermore, iron limitation and inactivation of fur affected the expression of a subset of genes, involved in energy and carbon metabolism. Among them the most highly induced was a flavodoxin encoding gene. In conclusion, these results show that the strict anaerobe *C. acetobutylicum* senses and responds to availability of iron on multiple levels using a sophisticated system that employs Fur.

PSV006

Improved Glucosamine Utilization by *Corynebacterium glutamicum* and its application for L-Lysine production

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Corynebacterium glutamicum is a Gram-positive soil bacterium used for the development of biotechnological processes to produce amino acids, organic acids and alcohols. To reduce production costs the application of inexpensive renewable carbon sources like starch hydrolysates and molasse is preferred. To explore alternative and sustainable carbon sources for biotechnological processes this contribution focuses on uptake and catabolism of glucosamine, a monomeric building block of the abundant natural polymer chitin.
Utilization of amino sugars by *C. glutamicum* has not been investigated, so far. Maximum growth rates of the wild type strain, *C. glutamicum* ATCC13032, with on glucosamine as sole carbon source reach less than 50 % of the rates observed during cultivation on glucose, fructose or sucrose. Employing a directed evolution approach, we isolated a mutant strain that overcame this growth limitation. Microarray analysis revealed an up-regulated expression of the *nagAB*-operon encoding glucoseamine-6P-deaminase NagB required for glucosamine catabolism. Indeed enzymatic activity of NagB was significantly increased in mutant cells compared to wild type strain. Reporter gene assays using transcriptional fusions of the wild type and the mutant *nagAB* promoter with a promoterless *gfp* gene showed that the increased expression level of the *nagAB* operon is caused by a nucleotide exchange in the promoter.
In addition, here we show that import of glucosamine is catalyzed by the phosphotransferase system (PTS). Interestingly, the glucose specific EII permease of PTS mediates the translocation and concomitant phosphorylation of glucosamine, as well. However, the k_m values of glucose and glucosamine import are considerably different; 15 μM, 340 μM respectively. This results in a successive consumption of both substrates that compete for the same transporter.
Applying this knowledge of import and catabolism of glucosamine we demonstrated that plasmid-encoded overexpression of the *nagB* gene in a L-Lysine producing strain of *C. glutamicum* improves glucosamine utilization. We observed almost the same product yield and productivity compared to glucose as sole carbon source. Therefore, a significant step to utilize chitin hydrolysates for amino acid production has been made.

PSV007

Characterization of biotin protein ligase from *Corynebacterium glutamicum*: enzymatic analysis, physiological role and biotechnological application

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Corynebacterium glutamicum is a biotin auxotrophic bacterium that is used for large-scale production of amino acids, especially of L-glutamate and L-lysine. It is known that biotin limitation triggers L-glutamate production and that L-lysine production can be increased by enhancing the activity of pyruvate carboxylase, one of two biotin-dependent proteins of *C. glutamicum*. A fragmentary biotin synthesis pathway, including the genes *bioA*, *bioD* and *bioB*, but lacking *bioF*, is encoded in the genome of *C. glutamicum* along with a gene (cg0814) annotated to code for putative biotin protein ligase BirA[1]. In *E. coli*, the biotin genes are regulated by a bifunctional BirA protein, which is active as biotin-protein ligase and as transcriptional repressor of the bio-genes[2]. BirA from C. glutamicum lacks an N-terminal DNA-binding domain and is not regulating biotin metabolism as shown here by transcriptome analysis. In order to analyse biotin protein ligase activity of the BirA from *C. glutamicum*, a discontinuous enzyme assay was established. A 105aa peptide corresponding to the carboxyterminus of the biotin carboxylase/biotin carboxyl carrier protein subunit AccBC of the acyl CoA carboxylases from *C. glutamicum* was used as acceptor substrate. Biotinylation of this biotin acceptor peptide was revealed with crude extracts of a strain overexpressing the birA gene and was shown to be ATP dependent. Thus, *birA* from *C. glutamicum* codes for a functional biotin protein ligase (EC 6.3.4.15). The *birA* gene was overexpressed and the resulting biotin protein ligase overproduction increased the level of the biotin-containing protein pyruvate carboxylase and entailed a significant growth advantage in glucose minimal medium. Moreover, *birA* overexpression improved L-lysine production by a model producer strain and resulted in a two-fold higher L-lysine yield on glucose as compared to the control strain.

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PSV008

Physiological effects of disrupting the acetate and acetone formation pathways in *Clostridium acetobutylicum*

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Clostridial acetone-butanol-ethanol (ABE) fermentation is a natural source for microbial n-butanol production and regained much interest in academia and industry in the past years. Due to the difficult genetic accessibility of *Clostridium acetobutylicum* and other solventogenic clostridia, successful metabolic engineering approaches are still rare. In this study, a set of five knock-out mutants with defects in the central fermentative metabolism were generated using the ClosTron technology, including the construction of targeted double knock-out mutants of *C. acetobtuylicum* ATCC 824. While disruption of the acetate biosynthetic pathway had no significant impact on the metabolite distribution, mutants with defects in the acetone pathway, including both acetoacetate decarboxylase- (Adc) and acetoacetyl-CoA:acyl-CoA transferase- (CtfAB) negative mutants, exhibited high amounts of acetate in the fermentation broth. Distinct butyrate increase and decrease patterns during the course of fermentations provided experimental evidence that butyrate, but not acetate, is re-assimilated via an Adc/CtfAB-independent pathway in *C. acetobutylicum*. Interestingly, combining the adc and *ctfA* mutations with a knock-out of the phosphotransacetylase- (Pta) encoding gene, acetate production was drastically reduced, resulting in an increased flux towards butyrate. Except for the Pta-negative single mutant, all mutants exhibited a significantly reduced solvent production in pH-uncontrolled batch fermentations as compared to the wildtype.

PSV009

4-Hydroxybutyryl-CoA dehydratase, a radical enzyme in metabolic pathways of anaerobic Bacteria and Archaea

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4-Hydroxybutyryl-CoA dehydratase (AbfD) was discovered in the fermentation of 4-aminobutyrate to ammonia, acetate and butyrate in *Clostridium aminobutyricum* [1]. Recently, this radical enzyme has been also identified in two new CO_2 fixation pathways in Archaea [2]. The [4Fe-4S] cluster and FAD containing AbfD catalyzes the oxygen sensitive and chemical difficult dehydration of 4-hydroxybutyryl-CoA to crotonyl-CoA, since the unactivated β-proton has to be removed ($pK \sim 40$). The successful production of variants of recombinant AbfD in *Escherichia coli* demonstrated that all highly conserved amino acids around the active center are essential for activity. Surprisingly, the low residual activity of the Y296F variant (<1%) suggested that a tyrosine radical may be involved in catalysis. This radical is probably formed upon substrate binding, because the substrate as well as its analogues butyryl-CoA and even CoA induce a partial one-electron reduction of the $[4Fe\text{-}4S]^{2+}$ clusters and FAD whereby Y296 might act as donor. Thus FAD-semiquinone rather than FAD could be oxidant and base to convert the enolate to an allylic ketyl radical that expels the hydroxyl group [3]. Experiments with 4-hydroxy[2-$^2H_2$]butyrate and 4-hydroxy[3-$^2H_2$]butyrate as substrates in vivo and in vitro indicated that neither the α- nor the β-proton is used to form the methyl group of crotonyl-CoA [4].

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PSV010

Characterization of a novel anaerobic steroid C25 dehydrogenase (DMSO reductase family) in Sterolibacterium denitrificans

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Cholesterol is one of the most abundant steroid compounds in nature. This ubiquitous steroid and related cyclic compounds are recalcitrant to degradation owing to their low solubility in water, complex ring structure, the presence of quaternary carbon atoms, and the low number of functional groups. Nevertheless, cholesterol can serve as substrate for microbial growth. In aerobic metabolism, oxygenases use reactive molecular oxygen as co-substrate of oxygenases to hydroxylate the molecule and cleave the steran ring system. Obviously, anaerobic metabolism must substitute oxygenase catalyzed steps by O2-independent reactions, e.g. by hydroxylation. We show that one of the initial reactions of anaerobic cholesterol metabolism in the betaproteobacterium Sterolibacter denitrificans is catalyzed by an novel enzyme that hydroxylates the tertiary C25 atom of the side chain in oxygen-independent manner forming a tertiary alcohol. This steroid C25 dehydrogenase belongs to the dimethylsulfoxide dehydrogenase molybdoenzyme family, the closest relative being ethylbenzene dehydrogenase. It is a heterotrimer, which is located at the periplasmic side of the membrane and contains 1 molybdenum cofactor, 5 [Fe4S4] clusters, and 1 heme b. Analysis of the draft genome of the organism revealed several genes coding for related enzymes that likely operate instead of oxygenases in anoxic steroid metabolism.

PSV011

Investigating the reaction mechanism of crotonyl-CoA carboxylase/reductase: Exploring the bio(techno)logical potential of reductive carboxylation.

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Crotonyl-CoA reductase/carboxylase (CCR) catalyzes the reductive carboxylation of crotonyl-CoA with NADPH as co-factor yielding ethylmalonyl-CoA. If no CO_2 is present crotonyl-CoA is reduced to butyryl-CoA [1]. CCR and its homologs provide building blocks (e.g. ethylmalonyl-CoA, chloroethylmalonyl-CoA, hexylmalonyl-CoA, etc.) in polyketide (antibiotic) biosynthesis, which makes them attractive candidates for introducing modifications into the polyketide backbone [2]. However, to be able to utilize CCR in biotechnology, a good understanding of the mechanism is desired to facilitate enzyme engineering attempts.
In order to study the mechanism of CCR the crystal structure of CCR was overlaid with the homologous structure of a well characterized horse liver short chain alcohol dehydrogenase to identify amino acid residues important in catalysis and CO_2 binding. The contribution of several amino acids on carboxylation and reduction reaction was tested by site directed mutagenesis. Apparently, the enzyme has a hydrophobic cavity to promote the carboxylation reaction over the reduction.
Further investigation on the reaction mechanism of CCR identified the formation of a transient intermediate. This intermediate was further characterized spectrophotometrically and by NMR, indicating that it exist as a free species (non-enyzme bound) in the absence of CO_2. This observation challenges the mechanism of reductive carboxylation as it was originally proposed [1].

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PSV012

The unconventional octahaem cytochrome *c* MccA is the terminal reductase of *Wolinella succinogenes* sulfite respiration

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Assimilatory and dissimilatory sulfite reductions are key reactions of the biogeochemical sulfur cycle and several distinct sirohaem-containing sulfite reductases have been characterized in the past, for example in sulfate-reducing bacteria and archaea. Here, we describe that the Epsilonproteobacterium *Wolinella succinogenes* grows by respiratory sulfite respiration (yielding sulfide) using formate as electron donor [1].
Sulfite is reduced in the periplasm by the octahaem cytochrome *c* MccA which represents a new class of sulfite reductase. The synthesis of MccA in *W. succinogenesis* induced by the presence of sulfite (but not by thiosulfate or sulfide). MccA represents an unusual multihaem cytochrome *c* as one of its eight haem groups is bound via a unique $CX_{15}CH$ haemc binding motif [2,3]. The phenotypes of numerous site-directed MccA variants underlined the structural importance of this motif.
Several open reading frames of the *mcc* gene cluster were individually inactivated on the *W. succinogenes* genome and characterization of the corresponding mutant strains indicated that the predicted iron-sulfur protein MccC, the putative quinol dehydrogenase MccD (a member of the NrfD/PsrC family [4]) as well as a peptidyl-prolylcis-transisomerase, MccB, are involved in sulfite respiration. Similarly, the open reading frames *mccRS* which encode a regulatory two-component system were found to be required for MccA induction by sulfite.
Based on the results, a sophisticated model of epsilonproteobacterial sulfite respiration will be presented.

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PSV013

Localization and regulation of PHB granules in *Synechocystis sp*. PCC 6803

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Polyhydroxyalkanoates (PHA) are organic polyesters composed of (R)-3-hydroxy fatty acids which are synthesized by many bacteria as a carbon and energy storage material under unbalanced nutrient and energy availability. PHAs are deposited intracellularly as insoluble spherical inclusion called PHA granules, which consist of a polyester core surrounded by a phospholipid layer with attached proteins. One of these proteins is the PHA synthase, the key enzyme of PHA biosynthesis, which catalyses the formation from (R)-3-hydroxyacyl-CoA precursors. Only little is known about the regulation and biogenesis of PHA accumulation in cyanobacteria. We investigate the granule self-assembly process, the function of granule-associated proteins and the regulation of the PHB accumulation in *Synechocytis* PCC 6803. We applied different fluorescence dyes, as well as GFP-PHA synthase fusion proteins to study the early PHA granule formation. With these tools we investigate, whether this process is located at the cytoplasmatic membrane. We analyzed the proteome of purified PHA granules and identified new putative granule-associated proteins. To gain insight into the regulation of PHA synthesis, we investigate mutants, which are impaired in PHA accumulation. Here we show that the NADPH pool is crucial for the PHA accumulation.

PSV014

Biosynthesis of (Bacterio)chlorophylls: ATP-Dependent Transient Subunit Interaction and Electron Transfer of Dark-operative Protochlorophyllide Oxidoreductase

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The biosynthesis of (bacterio)chlorophylls is fundamental for the primary production on earth. Reduction of the fully conjugated ring system of protochlorophyllide results in the common core ring architecture which is characteristic for all (bacterio)chlorophylls. Dark-operative protochlorophyllide oxidoreductase (DPOR) is a multi-subunit enzyme employing nitrogenase-like catalysis for the chemically difficult two electron reduction of ring D.
During ATP-dependent DPOR catalysis the homodimeric $ChlL_2$ subunit carrying a [4Fe-4S] cluster, transfers electrons to the corresponding heterotetrameric catalytic subunit $(ChlN/ChlB)_2$ which also possesses a redox active [4Fe-4S] cluster. To investigate the transient interaction of both subcomplexes and the resulting electron transfer reactions, the ternary DPOR enzyme holocomplex comprising subunits ChlN, ChlB and ChlL was trapped as an octameric $(ChlN/ChlB)_2(ChlL_2)_2$ complex after incubation with non hydrolyzable ATP analogs. A nucleotide-dependent switch mechanism triggering ternary complex formation and electron transfer was concluded.
The crystal structure of the catalytic $(ChlN/ChlB)_2$ complex of DPOR from the cyanobacterium Thermosynechococcus elongatus was solved at a resolution of 2.4 Å. Subunits ChlN and ChlB exhibit a related architecture of three subdomains built around a central, parallel ß-sheet surrounded by α-helices. The $(ChlN/ChlB)_2$ protein revealed a [4Fe-4S] cluster coordinated by an oxygen atom of an aspartate residue alongside three common cysteine ligands. Two substrate binding sites enriched with aromatic residues for coordination of the protochlorophyllide substrate molecules are located at the interface of each ChlN/ChlB half-tetramer. The complete octameric $(ChlN/ChlB)_2(ChlL_2)_2$ complex of DPOR was modeled based on the obtained structure and earlier functional studies. The electron transfer pathway via the various redox centers of DPOR to the substrate was reconstructed.

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PSV015

Carbon disulfide hydrolase: a new enzyme for CS_2 conversion in acidothermophilic microorganisms

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Acidophilic, thermophilic Archaea that live in mudpots of volcanic eocsystems obtain their energy from the oxidation of sulfur compounds such as carbon disulfide and hydrogen sulfide, thereby creating an extremely acidic environment with pH values as low as 1. A hyperthermophilic Acidianus strain A1-3 was isolated from the fumarolic, ancient sauna building at the Solfatara volcano (Naples, Italy). It was shown to rapidly convert CS_2 into H_2S and carbon dioxide (CO_2), but so far little was known about the modes of action and the evolution of the enzyme(s) involved. In this study we elucidated the structure, the proposed mechanism and evolution of the isolated CS_2 hydrolase from Acidianus A1-3. The enzyme monomer displayed a typical b-carbonic anhydrase fold and active site, yet CO_2 was not one of the typical substrates. Large carboxy- and amino-terminal arm extensions, and an unusual hexadecameric catenane oligomer were apparent in the enzyme. These structure features resulted in the blocking of the usual entrance to carbonic anhydrase active sites, and the formation of a single 1,5 nm long, highly hydrophobic tunnel that functions as a specificity filter. The tunnel determines the enzyme's substrate specificity for CS_2. The transposon sequences that surround the gene encoding this CS_2 hydrolase point to horizontal gene transfer as a mechanism for its acquisition during evolution. Our results show how the ancient b-carbonic anhydrase, which is central to global carbon metabolism, was transformed by divergent evolution into a crucial enzyme in CS_2 metabolism.

Smeulders MJ, Barends T, et al (2011) Evolution of a new enzyme for carbon disulphide conversion by an acidothermophilic archaeon. Nature 478(7369):412-416 doi:10.1038/nature10464

PSV016

A promiscuous archaeal ATP synthase concurrently coupled to Na^+ and H^+ translocation

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Cytochrome-containing methanogenic archaea are one of the very few organisms that generate a primary proton- as well as a primary sodium ion gradient during their metabolism (1). Thus, the critical question is how both ion gradients are used for the synthesis of ATP. Since only one ATP synthase (A_1A_O type) is expressed, the enzyme may use both ions as coupling ion, or the sodium ion gradient is converted to a secondary proton gradient via Na^+/H^+ antiporter or vice versa (2). We have addressed this long standing question using energetically intact inside out membrane vesicles of *Methanosarcina acetivorans*. Our results show that the A_1A_O ATP synthase translocates both ions, H^+ and Na^+, simultaneously under physiological conditions. To further elucidate this phenomenon, we used free-energy molecular simulations to analyse the ion-selectivity of the ion-binding site of the subunit *c*. This appears to have been tuned via amino-acid substitutions allowing the usage of H^+ and Na^+ under physiological conditions. The adaptation of the binding site could be an adaptation to use the heterogeneous ion gradient established during methanogensis.

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PSP001

A tool for online measurement of the intracellular pH in *Corynebacterium glutamicum*

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The soil bacterium *C. glutamicum* is a well established organism for industrial amino acid production. In its natural habitat as well as during large scale fermentation, *C. glutamicum* is exposed to significant changes of the external pH. The limited mixing capacity, pH regulation by addition of acids or NH_3 as well as elevated CO_2 concentrations at high cell densities contribute to transient fluctuations of the external pH. It is considered that most bacteria perform efficient pH homeostasis in order to maintain the structural and functional integrity of cellular macromolecules but, it is unknown for almost all bacteria to what extent the internal pH can vary and how fast the adjustment of the internal pH is achieved upon external shifts. Additionally, the major players of pH regulation and the energetic costs of pH homeostasis are unknown, although their impact on the efficiency of production processes seems to be obvious. In order to address these questions we established a method for online measurement

of pH_i *in vivo* using the pH sensitive GFP variant pHluorin[1]. The measurement is independent of the amount of (functional) dye, fully reversible, insensitive towards ionic strength or inhibitors and allows sampling rates of less than 5 seconds. We applied the newly established method in *C. glutamicum* to quantify internal pH variations ranging from pH 5 -7.5 upon acidification of the surrounding, to follow the cellular response in time and to look for major contributors to the export of protons under acidic stress conditions.

1. Miesenböck et al., 1998

PSP002

Phenylacetaldehyde is oxidized by two different enzymes in anaerobic *Aromatoleum aromaticum* - phenylacetaldehyde ferredoxin oxidoreductase and phenylacetaldehyde dehydrogenase

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The mesophilic denitrifying bacterium *Aromatoleum aromaticum* degrades phenylalanine under anaerobic conditions to benzoyl-CoA, the common intermediate in anaerobic aromatics degradation. The most interesting step in this pathway is the oxidation of phenylacetaldehyde to phenylacetate. Two enzymes have been identified to catalyze this step: (i) a phenylacetaldehyde ferredoxin oxidoreductase (AOR, encoded by gene *ebA5005*) and (ii) a phenylacetaldehyde dehydrogenase (Pdh, encoded by gene *ebA4954*).

Enzymes of the AOR family contain a tungsten cofactor and most described representatives play important roles in peptide fermentation in hyperthermophilic archaea. However, more and more of these enzymes are also found in anaerobic mesophilic bacteria. For example, *A. aromaticum* produces an AOR-type enzyme when grown anaerobically on phenylalanine as sole carbon source (1). This corresponds to a simultaneously induced phenylacetaldehyde ferredoxin oxidoreductase activity in the respective crude extracts. The enzyme has been highly enriched and the presence of tungsten has been confirmed by ICP-MS measurements. Since anaerobic growth of the cells is also dependent on molybdenum-containing nitrate reductase, *A. aromaticum* must be able to produce molybdo- and tungstoenzymes at the same time. The pathways for molybdenum- and tungsten-cofactor biosynthesis are thought to be similar at least up to the step of metal incorporation (2). At this point, the organism needs to discriminate between molybdenum and tungsten. The genome of *A. aromaticum* contains different genes coding for potential molybdenum- or tungsten-specific transport and incorporation proteins, whose functions will be assessed by knock-out mutants.

In addition to AOR, a dehydrogenase using both NAD and NADP as electron acceptors (Pdh) is involved in anaerobic phenylacetaldehyde oxidation. The enzyme has been enriched and identified by MS analysis as gene product of *ebA4954*, which is different from an originally annotated NAD-dependent enzyme for this reaction (gene product of *ebA5381*) (3). The protein is oxygen-insensitive. Its gene is currently being cloned to be overexpressed in *Escherichia coli* and a knock-out mutant in *A. aromaticum* is being generated. Additionally, native and recombinant Pdh will be biochemically characterized.

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PSP003

Central metabolic enzymes as main target of reactive oxygen species in bacteria

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Since the appearance of photosynthetic cyanobacteria on planet earth, oxidative stress is a common problem for most bacteria and means the occurrence of ROS (reactive oxygen species) including hydrogen peroxide (H_2O_2), superoxide (O_2^-) or the hydroxyl radical (HO^-). In its natural habitat as well as during biotechnological applications *Corynebacterium glutamicum* is exposed to oxidative stress impacting the integrity of the membrane, proteins and DNA and therefore survival, growth and product yields. Remarkably, the response towards oxidative stress was addressed in several bacteria including *Escherichia coli* or *Deinococcus radiodurans* but is poorly understood in *C. glutamicum*.

In this contribution we focused on oxidative modifications of enzymes that are part of the central metabolism in *C. glutamicum* and discovered that fructose-1,6-bisphosphat aldolase (FBA) and isocitrate dehydrogenase (ICDH) are prominent targets. Both show a high degree and manifold ROS-dependent modifications of particular amino acid residues identified by Oxyblot™ and LC-MS/MS. For both, we found a correlation between the extent of oxidative modification and loss of enzyme activity under *in vitro* conditions and could prove the occurrence of these modifications *in vivo* as well. In contrast, other highly abundant proteins like phosphoglycerate kinase (PGK) are not modified to the same extent. In order to unravel the correlation between the modification of particular residues and the reduced activity, the enzymes of *C. glutamicum* were compared with proteins from *Deinococcus radiodurans*, *Streptococcus gordonii* and *Propionibacterium acnes* regarding sequence similarity and oxidative damage upon expression in *C. glutamicum*. Finally, a proteome wide analysis of oxidative modifications revealed that besides FBA and ICDH selected enzymes of the Glycolysis and the TCA appear to be more sensitive than other enzymes of various pathways. We will discuss common features of these enzymes that illustrate the multiplicity of oxidative protein damage in bacterial cells.

PSP004

Intracellular routes of iron delivery to modular redox enzymes

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Modular redox enzymes involved in energy conservation often comprise a large catalytic, a small electron-transferring and a membrane anchor subunit. Examples in anaerobically growing *Escherichia coli* include the [NiFe]-hydrogenases (Hyd), nitrate reductase (Nar) and the formate dehydrogenases (Fdh). The activity of these enzymes relies heavily on the iron sulfur [FeS] cluster-containing small subunit[1]. The main [FeS] insertion machinery is the Isc (iron sulfur cluster) system, in which IscU has a scaffold function and IscA and ErpA are trafficking proteins[2]. However, not only the redox enzymes themselves require [FeS] clusters, but also many of the maturation proteins involved in active site provision contain an [FeS] cluster. In addition, the active site of [NiFe]-Hyd contains a single iron atom. In order to elucidate the role of the Isc [FeS] cluster biogenesis machinery in the formation of these modular redox enzymes, knock-out mutants were used to monitor the respective enzyme activities and immunological methods were employed to analyze the subunit composition. The ErpA and the IscU components were indispensable for generation of active redox enzymes, while the dependence on IscA was only partial. *E. coli* synthesizes three membrane-bound Hyd enzymes with Hyd-1 and Hyd-2 functioning as hydrogen-oxidizing enzymes and Hyd-3 forming part of the hydrogen-evolving formate hydrogen lyase (FHL) complex[3]. The FHL complex was partially functional in an *iscA* mutant, while Hyd-1 and Hyd-2 activities were undetectable. This phenotype was found to be due to the absence of the respective small subunit. Processing of all large Hyd subunits, which correlates with insertion of active site iron, still occurred. Therefore, insertion of active site iron must be independent of the [FeS] machinery and involves further unknown components.

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PSP005

C-Type Cytochromes in Hydrogen Oxidation and Sulfur Reduction in the Hyperthermophilic Archaeon *Ignicoccus hospitalis*

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Ignicoccus hospitalis is a strictly chemolithotrophic and hyperthermophilic archaeon that grows by anaerobic hydrogen oxidation with sulfur as electron acceptor. *Ignicoccus* species are special among archaea because they possess and inner and outer membrane enclosing an intermembrane compartment, which is not similar to the periplasmic space of Bacteria. A_0A_1-ATPases and sulfur reductase/hydrogenase are localized in the outer but not in the inner membrane. Here we describe purification results with hydrogenase, sulfur reductase and electron-mediating multiheme *c*-type cytochromes.

The hydrogenase purified from *I. hospitalis* membrane fractions consisted of four subunits, Igni_1366-1369, when separated on SDS gels, which included the large and small NiFe hydrogenase subunits, the membrane anchor and the so far elusive Isp2 FeS subunit. The hydrogenase reduced viologen dyes (115-660 U/mg), 2,3,-Dimethylnaphthoquinone (12 U/mg) and the soluble multiheme c-type cytochrome Igni_0955 in a semi-quantitative assay. The sulfur reductase has specific activities of 10-12 U/mg in solubilized membrane fractions, however, this decreased rapidly upon further purification. No proteins were identified.

Besides Igni_0955, *I. hospitalis* contains a second soluble multiheme cytochrome *c*, termed Igni_1359. Both cytochromes were purified chromatographically 16 and 11-fold, respectively, to apparent

homogeneity. Both run as dimers in gel filtration chromatography but they also form higher aggregates in denaturing and non-denaturing electrophoresis. The absorption maxima were 552, 525, and 410 nm (reduced: 420nm) for Igni_0955 and 554, 521 and 409 nm (reduced 419 nm) for Igni_1369. Hemochrome spectra showed ≈ 7 hemes/subunit, while mass spectroscopy resulted in 8 hemes for both proteins in accordance with the prediction from sequence. Igni_1369 is one of the most abundant proteins in *Ignicoccus* cells (≈5%) but its function is unknown.
A survey of multiheme proteins in Archaea showed that they occur in some but not all of the Desulfurococcales, the Archaeoglobi and the Methanomicrobia, and in the species *Pyrobaculum calidifontis* and *Natrialba magadii*. An overview of the distribution of various types of c-type cytochromes in Archaea will be discussed.

PSP006

Studies on the interaction of the *O*-demethylase components of the anaerobe *Acetobacterium dehalogenans* using two-hybrid systems

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The anaerobe acetogen *Acetobacterium dehalogenans* utilizes the methyl group of phenyl methyl ethers, which are products of lignin degradation, as a carbon and energy source. The *O*-demethylation reaction in which the methyl group of the substrate is transferred to tetrahydrofolate is mediated by the key enzymes, the *O*-demethylases, in the methylotrophic metabolism. Different *O*-demethylases are induced in response to different phenyl methyl ethers formed upon fungal lignin degradation.
The *O*-demethylase complex consists of four enzymes: a methyltransferase I (MT I), a methyltransferase II (MT II), a corrinoid protein (CP) and an activating enzyme (AE). The methyl group is transferred from the phenyl methyl ether to the super-reduced corrinoid protein by MT I. The methylated corrinoid protein is subsequently demethylated and the methyl group is transferred to tetrahydrofolate by MT II. The inactivated form of the corrinoid protein, cob(II)alamin, which may be generated by inadvertent oxidation, is reduced by the activating enzyme in an ATP dependent reaction.
To catalyze the complete *O*-demethylase reaction, an interaction of at least three of the four proteins components is required. Protein-protein interactions were investigated using bacterial and yeast two-hybrid systems. First results indicate that CP, as methyl group carrier during the *O*-demethylation process interacts with all other proteins of the *O*-demethylase complex. This finding supports the crucial role of CP in the methylotrophic metabolism of *Acetobacterium dehalogenans*.

PSP007

Design of a bacterial electron transport module: Interaction of membrane-bound NiFe-hydrogenase with cytochromes *b* and *c*

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Many bacteria employ membrane-bound NiFe-hydrogenases (MBHs) that serve in hydrogen gas uptake and electron transport in anaerobic respiratory chains. MBHs possess a heterodimeric protein complex that contains the active site of hydrogen turnover and three iron-sulfur clusters. This entity is located at the periplasmic side of the membrane and linked to the membrane via a quinone-reactive dihaem cytochrome *b*. In contrast, soluble heterodimeric NiFe-hydrogenases from, for example, sulfate reducers are periplasmic enzymes that interact with multihaem cytochromes *c*.
The MBH complex of the Epsilonproteobacterium *Wolinella succinogenes* (HydABC) is the key enzyme of anaerobic respiration using hydrogen gas as electron donor. The enzyme is anchored to the membrane by both the dihaem cytochrome *b* HydC and a C-terminal transmembrane helical region of the iron-sulfur subunit HydA [1,2]. In the absence of both anchors, active hydrogenase was found almost exclusively in the periplasmic cell fraction [1].
The aim of this work was to identify amino acid residues involved in HydA-HydC interaction. Furthermore, the ability of *W. succinogenes* MBH to reduce cytochromes *c* was investigated using purified HydABC or cell fractions containing the periplasmic HydAB complex. Advised by co-evolutionary dependency studies based on information theory, engineering of HydAB was performed in order to optimize cytochrome *c* reduction by hydrogen gas, thus designing a novel periplasmic electron transfer network in *W. succinogenes*.
[1] Gross *et al.* (1998) *Arch Microbiol* 170: 50-58
[2] Gross *et al.* (2004) *J Biol Chem* 279: 274-281

PSP008

The methylotrophic metabolism of *Desulfitobacterium* spp.

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Desulfitobacterium spp. are strictly anaerobic bacteria first isolated from environments contaminated with halogenated compounds. In 2004, it was shown, that at least two strains of *Desulfitobacterium hafniense* (DCB-2 and PCE-S) are able to use phenyl methyl ethers, which are degradation products of lignin, as electron donors. By then, only acetogens had been reported to convert these compounds under anoxic conditions. In contrast to acetogenic bacteria, *Desulfitobacterium hafniense* is not able to use CO_2 as electron acceptor.
We currently investigate the metabolic pathways involved in the phenyl methyl ether consumption of *Desulfitobacterium hafniense* DCB-2. Key enzymes are the *O*-demethylases, inducible enzyme systems first described for acetogens. On the basis of the genome sequence 17 putative *O*-demethylase operons were identified. Recent studies concentrate on the heterologous expression of putative *O*-demethylase genes and the characterization of the corresponding gene products.

PSP009

Temporal and spatial effects of adaptation, a new mechanism relying on posttranslational modification of key enzymes in degradative microorganisms

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A vast spectrum of organic chemicals is steadily released to the environment by the industry and consumers. Despite the xenobiotic character of these chemicals, the main process responsible for mitigating their impact is pollutant degradation by microorganisms. The capability of microorganisms to adapt to environmental pollutants and to couple their degradation to growth has been attributed to genetic mechanisms like mutation and recombination of genes. However, other mechanisms may also explain adaptative responses of microorganisms. Herein, we present evidence for a mechanism improving the activity of degradative enzymes by posttranslational modification.
The soil bacterium *Delftia acidovorans* MC1 was used; it degrades phenoxyalkanoate herbicides like 2,4-dichlorophenoxyacetate (2,4-D) and (*RS*)-2-(2,4-dichlorophenoxy-)propionate ((*RS*)-2,4-DP). Key enzymes for the initial degradation step are α-ketoglutarate-dependent dioxygenases, which determine the microorganism's substrate specificity, e.g. the (*R*)-2,4-DP/α-ketoglutarate dioxygenase (RdpA) attacks the R-enantiomer of (*R*)-2,4-DP but not 2,4-D. The latter is cleaved by 2,4-D/- and (*S*)-2,4-DP/α-ketoglutarate dioxygenases (TfdA and SdpA, respectively). We studied adaptation in long-term cultivation experiments with mutant strains bearing only RdpA. Noteworthy, cultivation in the presence of (*R*)-2,4-DP and 2,4-D led to improved degradation of 2,4-D (K_m) and its utilization for biomass formation. This was accompanied by a change in the enzyme pattern, as made visible by 2D gel electrophoresis, showing line-ups of RdpA forms varying in their *pI* and number. Since there is only one *rdpA* gene in the genome of *D. acidovorans* and no mutations were found, posttranslational modification is a likely explanation for the appearance of RdpA variants. Particularly plausible are charge relevant carbonylation reactions since they alter the proteins' *pI*, as observed in our study. Carbonylation is induced by reactive oxygen species (ROS), which are known side products of oxygenase reactions which, in turn, cause carbonylation of the enzyme itself and other proteins in its vicinity. Carbonyl groups were identified through Western blotting via their specific reactions with dinitrophenylhydrazine. Our study of *D. acidovorans* adaptation was extended to a two-dimensional microfluidic pore network, which simulates subsurface pore spaces. Here, initial growth on (*R*)-2,4-DP and adaptation on 2,4-D was observed via reflected DIC microscopy in the pore network. Effluent collected during adaptation is currently analyzed for the appearance of RdpA variants. Our research provides insight into adaptational capabilities of microbial strains in biotopes with limited genetic diversity, and defines growth properties at limiting substrate concentrations which are relevant for treatment of contaminants in soil and groundwater.

PSP010

Crystal structure of the electron-transferring flavoprotein (Etf) from *Acidaminococcus fermentans* involved in electron bifurcation

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Aerobic organisms use electron-transferring flavoprotein (Etf) as electron acceptor for the oxidation of acyl-CoA to enoyl-CoA. The reduced form of Etf is then reoxidized by quinone at the inner mitochondrial membrane of eukaryotes or at the cytoplasmic membrane of bacteria. The structure of the human heterodimeric Etf (βγ) revealed three domains, two of which are formed by the β-subunit (I and II) and one by the γ-subunit (III). β-FAD located at the surface of domain II interacts with acyl-CoA dehydrogenase. The center of domain III contains AMP with an enigmatic function. The interface between domains II and III appears to be flexible due to absence of secondary structures [1].
Anaerobic bacteria synthesize butyrate via the NADH-dependent reduction of crotonyl-CoA to butyryl-CoA mediated by butyryl-CoA dehydrogenase and Etf. This exergonic reaction is coupled to the endergonic reduction of ferredoxin by NADH, a process called electron bifurcation [2]. Whereas in *Clostridium klyuveri* [3] and *Clostridium tetanomorphum* butyryl-CoA dehydrogenase and Etf form a tight complex, in *A. fermentans* both components separate during purification. Recombinant Etf from *A. fermentans* produced in *Escherichia coli* was crystallized and its structure has been solved. The structure is closely related to that of the human Etf, but AMP is replaced by a second γ-FAD. We propose that NADH reduces γ-FAD to γ-$FADH^-$. Then β-FAD switches towards γ-$FADH^-$ and takes one electron to yield γ-$FADH^-$ and β-$FAD^{\cdot-}$. Whereas β-$FAD^{\cdot-}$ is stabilized by the flavodoxin-like domain II and transfers the electron further to the dehydrogenase, the remaining highly reactive γ-$FADH^-$ immediately reduces ferredoxin. Repetition of this process results in the reduction of 2 ferredoxins and one crotonyl-CoA by 2 NADH. The reduced ferrdoxins may give rise to H_2 or to $\Delta\mu H^+/Na^+$ via a membrane bound NAD-ferredoxin oxidoreductase also called Rnf.

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2. Herrmann G, Jayamani E, Mai G & Buckel W (2008) J Bacteriol 190, 784-791
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PSP011

Nitrous oxide turnover in the nitrate-ammonifying Epsilonproteobacterium *Wolinella succinogenes*

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Global warming is moving more and more to the public consciousness. Besides the commonly mentioned carbon dioxide, nitrous oxide (N_2O) is one of the most important greenhouse gases and accounts for about 10% of the anthropogenic greenhouse effect.
In the environment N_2O is produced, for example, by nitrifying and denitrifying microbial species. On the other hand, some respiratory nitrate-ammonifying Epsilonproteobacteria are able to reduce nitrous oxide to dinitrogen via an unconventional cytochrome *c* nitrous oxide reductase (*c*NosZ). The energy metabolism of one of these bacteria, *Wolinella succinogenes*, has been characterized thoroughly in the past. The cells are able to use either formate or hydrogen gas as electron donors together with typical terminal electron acceptors like, for example, fumarate, nitrate, polysulfide or nitrous oxide. Despite utilizing nitrous oxide, it is not known if these cells are producing N_2O in substantial amounts during energy substrate turnover or if they are acting only as N_2O sinks.
The cytochromecnitrous oxide reductase of *W. succinogenesis* encoded by the first gene of the *nos* gene cluster together with a unique electron transport system that is predicted to connect the menaquinone/menaquinol pool with *c*NosZ. The involved electron transfer chain may comprise a menaquinol dehydrogenase of the unusual NapGH-type and the two monohaem cytochromes *c* NosC1 and NosC2. Corresponding in-frame gene deletion strains were constructed and characterized. Based on the results, a model of nitrous oxide turnover in *W. succinogenes* will be presented.

PSP012

Anaerobic *n*-hexane degradation in nitrate reducing strain HxN1

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The denitrifying Betaproteobacterium HxN1 grows on *n*-hexane [1] forming alkyl substituted succinates. The proposed pathway starts with the addition of *n*-hexane to fumarate with the exclusive abstraction of the pro-*S* hydrogen of *n*-hexane via a glycyl-radical enzyme catalysed reaction [1], yielding a mixture of (2*R*,1'*R*) and (2*S*,1'*R*)-1'-methylpentylsuccinates most likely as CoA-thioesters [2]. These intermediates are proposed to be degraded via intramolecular rearrangement to (4*R*)-(2-methylhexyl)malonyl-CoA and carboxyl group loss yielding (4*R*)-4-methyloctanoyl-CoA. Further degradation may occur via dehydrogenation and β-oxidation [3]. If (4*R*)-(2-methylhexyl)malonyl-CoA, synthesised by a novel method, and propionyl-CoA were incubated with cell-free extract of strain HxN1, MALDI-TOF mass spectrometry revealed formation of methylmalonyl-CoA and 2-methylhex-2-enoyl-CoA (β-oxidation product). Therefore, transcarboxylation (CO_2 exchange between substrates) occurs at the CoA thioester level as predicted, linking the degradation of 1-methylpentylsuccinate to the generation of succinate via methylmalonyl-CoA.

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2) Jarling R, Sadeghi M, Drozdowska M, Lahme S, Buckel W, Rabus R, Widdel F, Golding BT, Wilkes H (2011), Angew. Chem. in press.
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PSP013

Streptomyces coelicolor A3(2) Spores are Prepared for an Abrupt Shift from Aerobic Respiration to Anaerobic Respiration with Nitrate

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The filamentous actinobacterium *Streptomyces coelicolor* has a complex life cycle including growth as vegetative hyphae, generation of hydrophobic aerial hyphae and the production of exospores. Despite being an obligate aerobe *S. coelicolor* is able to reduce nitrate to nitrite, probably to help maintain a membrane potential during oxygen limitation. The genome of *S. coelicolor* has 3 copies of the *narGHJI* operon, each encoding a nitrate reductase (Nar) [1]. Nars are multi-subunit, membrane-associated enzymes that couple nitrate reduction to energy conservation. Each Nar enzyme is synthesized in *S. coelicolor* and is active in different phases of growth and in different tissues: Nar1 is active in spores; Nar2 is active in germinating spores and mycelium; while Nar3 is induced in the stationary phase correlating with the onset of secondary metabolism [2]. The Nar enzymes are therefore not redundant but rather appear to have distinct functions in the developmental program of the bacterium.
In this study we focused on nitrate respiration in resting spores. Freshly harvested spores of *S. coelicolor* wild type M145 could reduce nitrate at a significant rate without addition of an exogenous electron donor. However, an exogenous electron donor was required to measure the activity in crude extracts of spores. Moreover, activity could be visualized by direct staining after native PAGE. Analysis of defined knockout mutants demonstrated that Nar activity in spores was due exclusively to Nar1. By using a discontinuous assay to measure nitrite production by spores we could demonstrate that Nar1 was only capable of nitrate reduction in the absence of oxygen. Addition of oxygen immediately prevented nitrate reduction. Since Nar1 activity in whole spores showed a reversible dependence on anaerobiosis, this finding suggests that spores regulate either nitrate transport or Nar1 activity in response to oxygen. Notably, studies using protein synthesis inhibitors revealed that Nar1 is always present and active in resting spores.

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[2] Fischer *et al*. (2010) The obligate aerobe *Streptomyces coelicolor* A3(2) synthesizes three active respiratory nitrate reductases. Microbiology. 156(Pt 10):3166-79

PSP014

New insights into acetate and glycerol metabolism of *Schizosaccharomyces pombe*

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The fission yeast *Schizosaccharomyces pombe* has been a model organism of molecular biology for decades. However, little is known about its

physiology and the utilization of different carbon sources. In the present work, we investigated the glycerol/acetate co-consumption by fission yeast.
In contrast to other well-known yeasts like *Saccharomyces cerevisiae*, *S. pombe* is not able to use C2-compounds, such as ethanol or acetic acid as sole carbon source because the necessary enzymes of the glyoxylat cycle are missing. In 2010, Matsuzawa, et al. reported, that *S. pombe* is also unable to use glycerol as sole carbon source, which is in accordance with our results but cannot be explained up to now. In 2011, the simultaneous consumption of glycerol and acetate by fission yeast has been reported (Klement, et al., 2011). Therefore we composed a minimal media containing glycerol and acetate as sole carbon sources. The specific growth rate of *S. pombe* was determined as 0.11 h^{-1}. The biomass yield was 0.48 g CDW g $substrate^{-1}$ and the respiratory quotient (RQ) 1.05. No ethanol or other typical fermentation products were detected in the culture supernatant. These findings suggest that glycerol and acetate are co-consumed under completely respiratory conditions. This is a striking difference compared to other yeasts, e.g. *S. cerevisiae*, where glycerol is used in the fermentative processes for the production of bioethanol.
We performed experiments with ^{13}C-labeld acetate to gain a deeper knowledge of the substrate distribution throughout the entire central carbon metabolism. Our results show, that glycerol is used as precursor for glycolysis, gluconeogenesis and the pentose phosphate pathway. Acetate is metabolized via the tricarboxylic acid cycle (TCA) but glycerol also contributes to the acetyl-CoA pool. No transport of mitochondrial oxaloacetate (OAA) into the cytosol was detected. Specific labeling patterns of proteinogenic amino acids revealed, that amino acids derived from OAA are synthesized exclusively in the cytosol. Further work will concentrate on the identification of possible regulatory mechanisms to understand, why *S. pombe* does not utilize glycerol as sole carbon source.
Klement, T., Dankmeyer, L., Hommes, R., van Solingen, P. and Buchs, J. (2011). Acetate-glycerol cometabolism: Cultivating *Schizosaccharomyces pombe* on a non-fermentable carbon source in a defined minimal medium.J Biosci Bioeng.112, 20-25.
Matsuzawa, T., Ohashi, T., Hosomi, A., Tanaka, N., Tohda, H. and Takegawa, K. (2010). The gld1+ gene encoding glycerol dehydrogenase is required for glycerol metabolism in *Schizosaccharomyces pombe*.Appl Microbiol Biotechnol87,715-27.

PSP015

A miniaturized parallel bioreactor system for continuous cultivation studies on yeast

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Chemostat cultivation is a powerful tool for physiological studies on microorganisms. The cells are kept at a stable physiological steady state and manipulations of environmental parameters like aeration and substrate availability are possible. The disadvantages of this system involve a long cultivation time to achieve a steady state and high substrate consumption, which can be problematic if expensive substances are used, e.g. isotopically labeled compounds.
We report the construction and application of a set of parallel bioreactors with 10 ml working volume for continuous cultivation. A similiar system has already been described for *E. coli* (Nanchen, et al., 2006) but has not been adapted to yeast cultivation up to now. Hungate tubes are used as culture vessels and placed in a water bath to maintain 30°C cultivation temperature. The rubber septum is pierced by needles, one connected to a multichannel peristaltic pump for feeding fresh media. A second multichannel pump is used for constant removal of culture broth to keep the culture volume at 10 ml. Since the efflux pump rate is far in excess of the feeding rate it is also used to induce aeration by generating under pressure inside the culture vessel. Sterile, water-saturated air is sucked into the tube via the third needle. A magnetic stirrer bar (9 x 6 mm) at the bottom of the vessel allows proper mixing and boosts oxygen transfer compared to a purely bubbled system. Dissolved oxygen (DO) was constantly measured via optical DO sensors to ensure aerobic conditions. In addition the DO-concentration is a powerful indicator of the physiological state of the cells inside the bioreactor. Off-gas analysis is performed by means of mass spectrometry.
Our system can be applied for continuous cultivation of yeast cells in up to 8 parallel bioreactors. DO-concentration profiles clearly indicate the achievement of the steady state. Utilization of magnetic stirrer bars guarantees proper mixing prohibiting sedimentation of cells and permits the use of small aeration rates (1 vvm) which is beneficial for accurate off-gas analysis. We used this system to characterize the shift from respiratory to respiro-fermentative growth for *Schizosaccharomyces pombe* and performed cultivations with ^{13}C-labeled substrate to determine intracellular fluxes through the central carbon metabolism.
Nanchen, A., Schicker, A. and Sauer, U. (2006). Nonlinear dependency of intracellular fluxes on growth rate in miniaturized continuous cultures of Escherichia coli.Appl Environ Microbiol72,1164-72.

PSP016

Biochemical and kinetic analysis of the acidophilic *c*-type cytochrome thiosulfate dehydrogenase from different Proteobacteria

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The acidophilic tetrathionate-forming enzyme thiosulfate dehydrogenase was isolated from the purple sulfur bacterium *Allochromatium vinosum*[1] and the corresponding gene (tsdA, YP_003442093) was identified on the main *A. vinosum* chromosome (NC_013851) on the basis of the previously determined N-terminal amino acid sequence. Thiosulfate dehydrogenase is a periplasmic, monomeric 25.8 kDa *c*-type cytochrome with an enzyme activity optimum at pH 4.3. UV-Vis and EPR spectroscopy indicate methionine (strictly conserved M_{222} or M_{236}) and cysteine (strictly conserved C_{123}) as probable sixth distal axial ligands of the two heme irons in TsdA. In addition UV-Vis spectroscopy revealed a minor peak at 635 nm which was assigned to the iron high-spin state. The low intensity of this high-spin-marker indicates that only a small portion of hemes exists in 5-coordination. An EPR spectrum of TsdA supplemented with its natural electron donor thiosulfate showed that the high spin heme is completely reduced at pH 5.0 but not at pH 8.0, which corresponds with the enzymes optimum pH for activity. Furthermore we determined the redox potential of the hemes.
Genes homologous to *tsdA* are present in a number of α-, β-, γ- and ε-proteobacteria. The wide-spread occurrence of *tsdA* agrees with reports of tetrathionate formation not only by specialized sulfur oxidizers but also by many chemoorganoheterotrophs that use thiosulfate as a supplemental but not as the sole energy source. For further analysis of TsdA we chose the facultative chemolithoautotrophic well-established sulfur oxidizer *Thiomonas intermedia*[2], the chemoorganoheterotrophic *Pseudomonas stutzeri*, for which tetrathionate formation from thiosulfate had previously been reported [3] and the psychro and halotolerant heterotrophic *Psychrobacter arcticus*[4], for which sulfur-oxidizing capabilities have never been investigated. All three proteins were produced in *E. coli* and proven to be *c*-type cytochromes which exhibited high specific thiosulfate dehydrogenase activities.
[1] Hensen et al. (2006) Mol. Microbiol.62, 794-810
[2] Moreira and Amils (1997) Int. J. Syst. Bacteriol.47,522-528
[3] Sorokin et al. (1999) FEMS Microbiol. Ecol.30, 113-123
[4] Bakermans et al. (2006) Int. J. Syst. Evol. Microbiol.56, 1285-1291

PSP017

Effects of High CO_2 Concentrations on Typical Aquifer Microorganisms

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The sequestration of carbon dioxide into the deep underground is considered as one option to reduce the emission of carbon dioxide into the atmosphere. A leakage of carbon dioxide from a deep storage site into a shallow aquifer is one of the main concerns connected to the Carbon Capture and Storage (CCS) technology. For a proper risk assessment it is necessary to study the influence of high CO_2 concentrations, as a result of leakage, on microorganisms, occurring in shallow aquifers. Therefore, growth curves and survival rates for four ecophysiologically distinct model organisms, ubiquitous in shallow aquifers, were determined. CO_2 concentrations in the gas phase varied between approximately 0 (refers to no amendment of CO_2) to 80% for the aerobic strains Pseudomonas putida F1 and Bacillus subtilis 168 and roughly 0 to 100% CO_2 for the nitrate-reducing strain Thauera aromatica K172 and the sulfate-reducing strain Desulfovibrio vulgaris Hildenborough. Carbon dioxide that infiltrates a freshwater aquifer under oxidizing conditions and under atmospheric pressure will have an immediate impact on water chemistry, leading to a reduction in pH. In our experiments, the pH of the growth medium decreased for about one unit from seven to six after the addition of CO_2. To distinguish between effects caused by carbon dioxide and the influence of decreasing pH-values, parallel experiments without CO_2 addition and decreased pH were performed. The results showed that growth and viability of all four strains were reduced at high CO_2 concentrations (> 50%), however, the aerobic strains are more sensitive to CO_2 stress compared to the anaerobic strains. After experiments at ambient pressure, growth experiments with increasing CO_2 concentrations and increasing pressure from 1 to 5000 kPa were performed in self constructed pressure vessels to simulate conditions typically occurring in deep aquifers. The combination of pressure and high CO_2 concentrations reduced significantly the viability of all tested strains. These results give first information for a concrete risk evaluation of the CCS technology and potentially leakage-related microbiological changes in shallow aquifers.

PSP018

Screening for genes of *Staphylococcus aureus* that are involved in the formation of persister cells

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Persisters are phenotypic variants of bacterial cells among a genetically identical population. These slow- or non-growing (dormant) cells are tolerant to antibiotics and are formed both stochastically and in adaptation to adverse conditions. Persisters seem to be causative for the recalcitrance of chronic infections to antimicrobial therapy. Notably, the molecular mechanisms underlying this kind of dormancy largely remain unclear particularly in bacteria beyond *E. coli*. We aimed at identifying genes governing the formation of persister cells in *Staphylococcus aureus* using two different strategies.
First, a screen for mutants exhibiting elevated persister levels was set up. 1 to 3.5 kbp fragments of a genomic library of *Staphylococcus aureus* SA113 as well as *S. aureus* homologs of *E. coli* persister genes *dnaJ*, *glpD*, *umuC* and the toxin-antitoxin (TA) RNases *yoeB-sa1* and *yoeB-sa2* were cloned into plasmid pRAB11 for tetracycline inducible control. Upon expression in SA113, mutants that show a reduced growth rate and enhanced antibiotic tolerance will be isolated. Sequencing of respective DNA fragments may thus reveal new or verify suspect *S. aureus* persister genes.
A second approach aims at generating *S. aureus* strains with decreased persister levels. To this end, single and combinational deletion mutants of *S. aureus* TA loci are constructed. These include the three verified chromosomally encoded systems *mazEF*, *yefM-sa1*/*yoeB-sa1* and *yefM-sa2*/*yoeB-sa2*, as well as two further putative TA-loci identified by in silico analysis. To remove resistance markers from newly generated mutant strains we are establishing the use of the yeast derived Flp/FRT recombination system in staphylococci. Obtained strains will be examined for persister formation and decreased antibiotic tolerance would support the hypothesis that TA-systems are crucial for persister formation in staphylococci.

PSP019

Will not be presented!

PSP020

Denitrification pathway is essential for complete functional magnetosome crystals for magnetic orientation in *Magnetospirillum gryphiswaldense*

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Magnetosomes are unique bacterial organelles used by magnetotactic bacteria (MTB) to orient in the Earth's magnetic field. In the α-proteobacterium *M. gryphiswaldense* (MSR-1) magnetosomes are crystals of magnetite (Fe_3O_4) which are biomineralized within specific vesicles of the magnetosome membrane. Maximum magnetite synthesis occurs only at low oxygen concentrations and in the presence of nitrate, suggesting a potential metabolic link between denitrification and magnetite biomineralization. However, no genetic evidence has been available *in vivo*.
Here we reconstructed a complete pathway of denitrification from the genome of MSR-1, including gene functions for nitrate (*nap*), nitrite (*nirS*), nitric oxide (*norCB*), and nitrous oxide reduction (*nosZ*). By characterizing deletion mutants of all genes, we showed that all proteins are required for anaerobic growth. In addition, deletions of *norCB*,*nirS* and *nap* impaired magnetite synthesis. The loss of *norCB* caused shorter magnetosome chains in ammonium medium, suggesting that nitric oxide reduction is involved in magnetosome formation also in microaerobic aerobic respiration. Deletion of the *nap* operon resulted in fewer, smaller and irregular crystals not only during denitrification but also aerobic respiration, probably due to disturbed redox balance for magnetite synthesis. Magnetite induction experiments by iron addition in nonmagnetic WT and Δ*nirS* cells under reduced and oxidized conditions revealed that the, nitrite reductase NirS is likely involved in anaerobic magnetosome formation by oxidizing ferrous to ferric iron. This process probably takes places in the periplasm by providing electrons for nitrite reduction, and ferric iron may subsequently be transported into magnetosome vesicles for magnetite synthesis.
Altogether, we provide evidence that the denitrification pathway has a key role for magnetite biomineralization by participating in redox reactions. This also shows that in addition to the various essential and accessory functions encoded within the genomic magnetosome island, also genes outside that region are involved in synthesis of functional magnetosome particles.

PSP021

A blueprint of organohalide respiration: Functional genome analysis of *Sulfurospirillum multivorans*

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Some of the most frequently detected contaminants in groundwater are halogenated organic compounds. Among them, tetrachloroethene (PCE) is the most abundant one. Due to its inertness, PCE is not easily degradable and persistent under oxic conditions. However, several anaerobic bacteria are able to couple the reductive dechlorination of PCE and of other organohalides to energy conservation via electron transport phosphorylation. Therefore, this process is often referred to as organohalide respiration.
Here, we present the functional analysis of the complete genome sequence of *Sulfurospirillum multivorans*, an ε-proteobacterium capable of dechlorinating tetrachloroethene (PCE) to dichloroethene. The latter can be readily degraded to non-toxic compounds by aerobic microorganisms. The high metabolic versatility of *S. multivorans* is reflected in one of the largest genomes of the ε-proteobacteria, comprised of a single circular chromosome 3.1 Mbp in length and includes more than 3,200 open reading frames. Close to the region coding for the PCE dehalogenase, which is a corrinoid-containing Fe-S cluster enzyme, corrinoid biosynthesis genes are located, and surprisingly genes coding for an additional putative reductive dehalogenase. Furthermore, we identified genes coding for all components of an aerobic respiratory chain and the TCA cycle. Together with current results from growth experiments, the data point to the first known example of an organohalide respiring organism capable of thriving in microaerobic environments.
In order to fill in the gaps in the understanding of anaerobic biological dehalogenation, this genome sequence of a gram-negative organohalide respiring bacterium is a big step toward to the complete elucidation of an outstanding way of microbial energy conservation.
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PSP022

Genomic and transcriptomic insights into *Allochromatium vinosum* DSM 180^T with special focus on genes involved in dissimilatory sulfur metabolism

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Anoxygenic purple sulfur bacteria like the *Gammaproteobacterium Allochromatium vinosum*, a member of the *Chromatiaceae*, flourish wherever light reaches sulfidic water layers or sediments and often occur as dense accumulations in conspicuous blooms in freshwater as well as in marine aquatic ecosystems. Here they are major players in the reoxidation of sulfide produced by sulfate-reducing bacteria in deeper anoxic layers. The capability to oxidize reduced sulfur compounds is the central metabolic feature of *A. vinosum* during photolithoautotrophic growth. Light energy is used to transfer electrons from reduced sulfur compounds such as sulfide, polysulfide, thiosulfate, sulfur and sulfite to the level of the more highly reducing electron carriers $NAD(P)^+$ and ferredoxin for reductive carbon dioxide fixation.
Here, we present a set of features of the complete genome (Acc: CP001896.1) of *A. vinosum*, the first member of the *Chromatiaceae*, for which a complete genome sequence is available. The genome consists of a 3,526,903 bp chromosome and two plasmids of 102,242 bp and 39,929 bp, respectively, with a total number of 3,366 predicted genes. A global transcriptomic analysis was performed with a special focus on oxidative dissimilatory sulfur metabolism in *A. vinosum*. To this end, total RNA was isolated after photolithoautotrophic growth on sulfide, thiosulfate, sulfur or sulfite as electron donor and compared to total RNA extracted from cultures grown photoorganoheterotrophically on malate. Firstly, these experiments confirmed the increased expression of genes encoding proteins already known to be involved in oxidative sulfur metabolism. Among these are the *dsr* genes [1] including *dsrAB* for dissimilatory sulfite reductase and the *sgp* genes for the proteins of the sulfur globule envelope [2]. Secondly, we also detected a number of interesting candidate genes that are highly upregulated in the presence of reduced sulfur compounds. Among these are several genes encoding potential sulfur relay proteins predicted to reside in the cytoplasm. Notably, transcription of some genes appeared to be specifically increased on insoluble sulfur. One of these gene products belongs to the lipocalin family of proteins. Members of this

family have been reported to participate in transport of insoluble substrates in *Eubacteria* [3].

[1] Dahl, C. *et al*., 2005. J. Bacteriol. 187, 1392-1404
[2] Prange, A. *et al*., 2004. Arch. Microbiol. 182, 165-174
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PSP023

Genetic evidence for a second anaerobic monoterpene-activating enzyme in *Castellaniella defragrans*

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Monoterpenes are natural compounds with an annual emission rate of 0.127 - 0.480 Gt C into the atmosphere, thus nearly reaching methane emission rates. The huge production rate is reflected in a frequent utilization of monoterpenes by bacteria. Most probable number studies revealed that each denitrifying bacterium in forest soil had the capacity. In activated sludge, one of 150 denitrifiers can grow on monoterpenes. The biochemistry of anaerobic monoterpene utilization is currently investigated with Castellaniella defragrans, a betaproteobacterium. The anaerobic monoterpene degradation of *Castellaniella defragrans* exhibits unique enzyme activities, but is still not fully elucidated. Deletion mutants were created lacking the gene for the linalool dehydratase-isomerase (*ldi*) as well as for both *ldi* and geraniol dehydrogenase (*ged*). These enzymes catalyze *in vitro* reactions of the anaerobic β-myrcene metabolism, the hydration of myrcene to geraniol and the geraniol oxidation. In the deletion mutants, the genes were absent on the genomic as well as the transcriptomic level without causing polar effect on the adjacent ORFs. The physiological characterization exhibited a substrate-dependent phenotype. The activity of the linalool dehydratase-isomerase was required for growth on β-myrcene, an acyclic monoterpene, but not on cyclic monoterpenes, i.e. α-phellandrene or limonene utilization proceeded without the presence of the linalool dehydratase-isomerase. This indicates a second enzyme system in *Castellaniella defragrans* that activates unsaturated hydrocarbons with cyclic structure.

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PSP024

Toxin-Antitoxin Systems in *Staphylococcus equorum*

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Chromosomally encoded Toxin-Antitoxin (TA) systems are assumed to play an important role in physiological adaptation of bacteria to environmental stresses. In *Staphylococcus aureus* three such TA systems have been characterized so far: One encoded by the $mazEF_{sa}$ locus and two by the paralogous $yefM_{sa}$/$yoeB_{sa}$ genes. *S. equorum*, a food industry relevant, nonpathogenic organism has recently been sequenced and is currently being annotated. The goal of this work was to identify and characterize putative TA systems in *S. equorum*. An *in silico* analysis yielded a *mazEF* homologue with a high similarity to its *S. aureus* counterpart, including co-localization with the *sigB* locus and two *yefM/yoeB* systems. $mazF_{se}$ from *S. equorum* was cloned into an anhydrotetracycline (ATc) inducible vector and used to transform *E. coli* DH5α. Induction of $mazF_{se}$ led to a ten-fold reduction of the OD_{578} values and to a severe growth inhibition on solid media. Even more strikingly, the number of CFUs was about 100-fold decreased compared to uninduced cells. For further characterization, the $mazEF_{se}$ transcription start was mapped via 5'-RACE and MazFse-$(His)_6$ was purified through affinity chromatography. MazFse was incubated in vitro with MS2 phage RNA and the resulting fragments analyzed via primer extension. Findings suggest the same target sequence as elucidated for the *S. aureus* MazF homologue: 5' U^ACAU 3'. This recognition sequence is overrepresented in some genes' mRNAs, most notably in *rsbV*, encoding an anti-anti-sigma factor of σ^B , possibly regulating *sigB* expression. In addition, the putative TA toxin genes $yoeB_{se1/2}$ were cloned into arabinose inducible *E. coli* vectors. Overexpression of these genes leads to a growth defect and further work to characterize these candidates is in progress. Based upon these observations, the inspected loci in *S. equorum* are highly indicative of encoding three functional TA systems.

PSP025

Will not be presented!

PSP026

Growth by Anaerobic Sulphur Dismutation in Thermophilic Archaea and Bacteria

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Anaerobic dismutation (disproportionation) of elemental sulphur for energy conservation is not well known. A few mesophilic bacteria including *Desulfocapsa sulfoexigens* were identified so far [1]. The products are hydrogen sulphide and sulphate. Here we show that several cocultures of different novel thermophilic microorganisms grow by chemolithoautotrophic sulphur dismutation.

We collected environmental samples from hot springs (60° to 90° C) on the island of São Miguel (Açores). Enrichment cultures were incubated in a minimal salt medium with elemental sulphur as energy source and in serum bottles with defined gas phases (aerobic, CO_2/H_2, or CO_2) to establish sulphur-oxidising, reducing or dismutating conditions, respectively.

We obtained one sulphur-oxidising culture (*Acidianus brierleyi*) plus one coculture (similar to *Thermus scotoductus*, *Alicyclobacillus*). Two sulphur-reducing cultures (*Acidianus brierleyi*, *Thermoplasma acidophilum*) and one sulphur-reducing coculture (*Thermoanaerobacter sulfurophilus*, *Thermoanaerobacter brockii*) were discovered. Furthermore we got two cocultures, which grew by sulphur dismutation under CO_2 atmosphere. The sulphur-dismutating cultures grew to cell densities of 5 x 10^7 ml^{-1} within 7 days. The maximal H_2S production was 860 μM in 7 days. One of these cultures was microscopically homogeneous and grew at 60 °C and pH 1.5. 16S rDNA sequencing showed that it was 99% identical to *Thermoplasma acidophilum*. The second culture grew at 80°C and pH 4 and showed a mixture of a rod-shaped and a coccoid microorganism. The 16S rDNA presumably of the rod-shaped microorganisms was 97% identical to *Vulcanisaeta distributa*. The coccoid microorganism could not be assigned phylogenetically.

In a second approach an *Acidianus ambivalens* / *Sulfurisphaera* MC1 coculture was grown autotrophically at 70-80 °C at pH 2-3 under CO_2 atmosphere and elemental sulphur as energy source. The coculture showed a stable growth with a doubling time of 120 h and a maximal cell density of 1 x 10^8 ml^{-1}. The results suggest that anaerobic sulphur dismutation is a common mechanism of energy conservation in habitats of volcanic origin, where sulphur is abundant and anaerobic habitats occur frequently.

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PSP027

Biotechnological production of 1,3-propanediol (1,3-PD): Overexpression of 1,3-PD operon and stabilization of 1,3-PD dehydrogenase from Colombian *Clostridium* sp.

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The non-pathogen Colombian strains *Clostridium* sp. IBUN 13A and IBUN 158B are able to produce 1,3-propanediol (1,3-PD) from raw glycerol. With the aim of improving the 1,3-PD yield of these strains for industrial purposes, the genes involved in the reductive pathway leading from glycerol to 1,3-PD were analyzed. The three genes *dhaB1*, *dhaB2* and *dhaT* are located next to each other on the genome of the strain IBUN 13A, lacking promoter or terminator sequences in between them. This suggests that they are coexpressed as an operon (1,3-PD operon). In order to verify that they belong to the same transcriptional unit, the corresponding promoterless region was cloned in the vector pJet1.2 (Fermentas) under the control of a T_7 promoter. The resulting plasmid was transformed in *E. coli* BL21(DE3), where overexpression of the cloned genes can be induced with IPTG for Northern blot analysis of the resulting RNA.

The product of the *dhaT* gene from IBUN 13A, a 1,3-PD dehydrogenase, was characterized after cloning in the overexpression vector pET-28a(+) (Novagen®). From this plasmid, the DhaT enzyme was overproduced and anaerobically purified through Ni-NTA-agarose (Qiagen) and PD-10 (GE Healthcare) columns. The size of the active enzyme was determined by native PAGE with the help of the NativeMark™ protein standard (Invitrogen), using polyacrylamide gels with concentrations in the range of 6-18 %. Activity of the enzyme was determined with 1,3-PD concentrations from 0 to 300 mM. In this way, the K_m value was calculated and a linear region of the activity curve was found, which is used for 1,3-PD measurement in an enzymatic test recently established (Franz *et al*., 2011). To improve the stability of the isolated DhaT enzyme, a lyophilisation protocol was developed, which allows activity preservation for up to 70 days.

In order to overproduce all enzymes from the reductive pathway in IBUN 158B, the 1,3-PD operon of IBUN 13A was cloned into the backbone of the conjugational vector pMTL007C-E2 (Heap *et al*., 2010) under the control of the P_{fdx} promoter. The resulting plasmid can be conjugated in inactivation mutants of IBUN 158B with a protocol based on Cai *et al*. (2011) to improve their 1,3-PD yield.

Cai G., Jin B., Saint C. *et al*., 2011. J. Biotechnol., 155, 269-274.
Franz S., Montoya J., Montoya D. *et al*., 2011. BioSpektrum, Tagungsband zur VAAM-Jahrestagung 2011, 155.
Heap J., Kuehne S., Ehsaan M. *et al*., 2010. J. Microbiol. Methods, 80, 49-55.

PSP028

Molecular genetic examination of 1-(4-hydroxyphenyl)-ethanol oxidation in "*Aromatoleum aromaticum*" EbN1

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The anaerobic degradation pathway forp-ethylphenol via 1-(4-hydroxyphenyl)-ethanol and *p*-hydroxyacetophenone in "*Aromatoleum aromaticum*" EbN1 was recently proposed, based on specific metabolite and protein formation. Genes, encoding respective enzyme candidates, form a large operon-like structure on the chromosome of strain EbN1. Remarkably, this gene cluster contains two neighbouring genes for dehydrogenases, which may perform the oxidation of 1-(4-hydroxyphenyl)-ethanol to *p*-hydroxyacetophenone. Both dehydrogenases share similar identity (<36%) to (*S*)-1-phenylethanol dehydrogenase, catalyzing an analogous reaction in the anaerobic ethylbenzene degradation pathway. Thus, it is presently unknown which of the two dehydrogenases actually catalyzes the oxidation of 1-(4-hydroxyphenyl)-ethanol top-hydroxyacetophenone.
In this study, the recently established genetic system for strain EbN1 was applied to generate an unmarked deletion mutant lacking both dehydrogenase encoding genes (*chnA* and *ebA309*). Growth of the ΔchnA/ebA309 mutant with *p*-hydroxyacetophenone was essentially as in the wild type, demonstrating absence of polar effects due to the deletion as could also be verified by transcriptional analysis. In contrast, anaerobic growth with *p*-ethylphenol was pronouncedly slower and of lower yield (wild type: OD_{max} >0.3 vs. mutant: OD_{max} <0.15). This very slow conversion of *p*-ethylphenol may be attributed to *p*-ethylphenol methylhydroxylase, the initial enzyme of the pathway, since a homologous enzyme of *Pseudomonas putida* also slowly oxidizes 1-(4-hydroxyphenyl)-ethanol. To specify the catalyzing dehydrogenase, three *in trans* complementation mutant strains were generated containing either (i) both genes, (ii) only *chnA* or (iii) only *ebA309*. While the strains ΔchnA/ebA309::*chnA*/*ebA309* and ΔchnA/ebA309::*chnA* restored growth with *p*-ethylphenol (similar to the wild type), the *ebA309* complemented mutant did not. Hence, the dehydrogenase ChnA is predicted to catalyze the oxidation of 1-(4-hydroxyphenyl)-ethanol in strain EbN1.

PSP029

Subcellular localization and distribution of enzymes of the energy metabolism and the CO_2 fixation pathway in *Ignicoccus*

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Ignicoccus hospitalis is a hyperthermophilic, chemolithoautotrophic Crenarchaeum that fixes CO_2 via the dicarboxylate/4-hydroxybutyrate cycle [1]. Optimal growth occurs at 90°C and it develops a slightly irregular coccoid shape. Moreover, *Ignicoccus* cells exhibit an extraordinary ultrastructure. In addition to the cytoplasmic membrane, there is an outermost membrane, encasing a huge intermembrane compartment (IMC). The IMC encloses vesicles which are released from the cytoplasmic membrane and fuse with the outermost membrane [2]. *I. hospitalis* also serves as host for *Nanoarchaeum equitans*, the only isolate of the Nanoarchaeota so far that can be grown in the laboratory [3]. Another unique feature of *I. hospitalis* is the localization of its A_1A_O ATP-synthase. Immuno-labeled ultrathin sections confirmed that this enzyme complex is almost exclusively found in the outermost membrane of *I. hospitalis* cells, grown with and without *N. equitans* [4]. Moreover, significant labeling was detected at the contact site between *N. equitans* and *I. hospitalis*, implicating the hypothesis that either ATP or subunits of the ATP-synthase are directly transferred to *N. equitans*. Labeling of the cytoplasmic membrane and of vesicles was observed occasionally. Additionally, immunolabeling studies on ultrathin sections with antibodies directed against the Acetyl-CoA synthetase, an enzyme involved in the CO_2-fixation pathway, again showed its localization in association with the outermost membrane of *I. hospitalis*. We are interested in analyzing the distribution of these and further proteins involved in the energy- and the CO_2-metabolism of *Ignicoccus*. Therefore, we mainly use electron microscopical methods. For best structural preservation, cells were cultivated in capillary tubes, high-pressure frozen, freeze-substituted and embedded in Epon. Ultrathin sections were labeled with primary and secondary antibodies. Secondary antibodies were coupled to ultrasmall or 6nm gold particles. In order to estimate the spatial distribution of gold particles on ultrathin sections, 3D reconstructions of *Ignicoccus* cells are performed. Finally, first 3D models were obtained from serial sections labeled with antibodies generated against the A_1 head of the *I. hospitalis* ATP synthase and the Acetyl-CoA synthetase.

[1] Jahn *et al*.,2007, Huber *et al*.,2008
[2] Rachel *et al*.,2002
[3] Huber *et al*.,2002
[4] Küper *et al*., 2010
[5] This research was supported by grants from the DFG (Germany)

PSP030

Membrane-bound electron transport in *Methanosaeta thermophila*

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Methanogenic archaea are widely distributed and of major importance for global carbon turnover and the biotechnological production of biogas. Numerous genera are able to grow on methylated compounds or H_2/CO_2 whereas the ability to grow on acetate (aceticlastic methanogenesis) is restricted to only two genera. The facultative aceticlastic genus *Methanosarcina* is well investigated, whereas there is essentially nothing known about the energy conserving mechanisms of the obligate aceticlastic genus *Methanosaeta*. *Methanosaeta* species prevail in habitats with low acetate concentrations as are frequently encountered in many natural and also man-made habitats like biogas digesters. The genomes of three *Methanosaeta* (*Mt*.) species were published recently but surprisingly the genomes did not contain genes encoding known electron-input complexes used in the respiratory systems of other aceticlastic methanogens. The investigations presented here focus on three issues: (i) Is a membrane-bound heterodisulfide oxidoreductase system present in *Mt. thermophila*? (ii) What is the electron donor to the *Mt. thermophila* respiratory chain? (iii) What is the initial electron acceptor protein in the membrane?
As a result, ferredoxin was the only electron donor that interacted with the membrane. Reduced ferredoxin was the electron donor to a heterodisulfide oxidoreductase system and high activities of the ferredoxin: heterodisulfide oxidoreductase could be observed. The lack of Ech hydrogenase and the Rnf complex indicates that a novel type of ferredoxin-interacting enzyme is involved. Our hypothesis is that a truncated form of the $F_{420}H_2$ dehydrogenase (a NADH dehydrogenase I homologue) is responsible for ferredoxin turnover. This hypothesis is also supported by strong expression of the corresponding genes as determined by quantitative PCR.

PSP031

Gene expression analyses combined with bioinformatic assessment sheds light on the Ecophysiology of sulfatases in Marine Bacteria

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Rhodopirellula baltica $SH1^T$ was the first genome-sequenced organism in the PVC superphylum [1]. Surprisingly, a high number of genes encoding sulfatases was identified. Recent bacterial genome projects originating from marine environments revealed that high numbers of sulfatases may be rather common. Especially members of the PVC superphylum show high abundances [1, 2]. Questions derived from those remarkable occurrences of sulfatases have not yet been answered: What are the advantages of carrying so many genes of the same family? How well is the sulfatase equipment conserved among marine bacteria? Is it usable as an evolutionary or biogeographical marker? Is the function of a given sulfatase derivable from sequence comparison to known sulfatases? We performed a combined bioinformatic and experimental approach to get new insights into the superfamily of sulfatases in planctomycetes.

Expression of sulfatases was studied with R. baltica SH1^T grown on different sulfated polysaccharides. Transcriptome-wide gene expression studies applying a well-established microarray platform [3, 4] revealed a strong functional link between tested substrates and active sulfatases. Besides, further potential functions mediated by sulfatases could be deduced from the expression profiles. The transcriptomic approach was combined with a phylogenetic assessment of sulfatase genes found in eight draft genomes of cultured strains representing five different species of the genusRhodopirellula [5, 6]. More than 1100 sulfatase sequences revealed 172 clusters of orthologous and (rare) paralogous genes. Phylogenetic analysis of theRhodopirellula sulfatases resulted in 17 major groups, of which only six included sulfatases of known function as derived from the UniProtKB database. Considering potential applications in medicine and biotechnology, sulfatases can be considered a promising hotspot in future research relating to the physiologically diverse PVC superphylum.

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PSP032

Solvent tolerance in Pseudomonas sp. strain VLB120 - Biofilms vs. Planktonic Cells-

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One of the key bottlenecks in biocatalysis involving toxic and / or organic substances is the stability of the chosen host organism. In the recent years a couple of strains belonging to the *Pseudomonas* genus have been described showing specific properties interesting for the conversion of toxic reactants [1], such as high solvent tolerance, metabolic versatility, and a high metabolic capacity for redox cofactor regeneration [2]. Another interesting feature of several *Pseudomonas* species is their ability to form biofilms. Solvent tolerance in planktonic cells is highly related to the existence of RND efflux pumps, especially TtgGHI in *P. putida* DOT-T1E and SrpABC in *P. putida* S12 [3-4]. The tolerance phenomena in biofilm growing cells are in part attributed to the existence of extracellular polymeric substances (EPS). EPS are excreted by biofilm growing organisms and form a sticky frame work giving the biofilm its three dimensional structure [5].

Here, we compare different mechanisms of *Pseudomonas* sp. strain VLB120 responsible for the excellent solvent tolerance of this strain [6-7] in planktonic growing cultures as well as in biofilm growing cells with the aim to obtain a stable solvent tolerant phenotype for redox biocatalysis with toxic reactants.

Regarding planktonic cells, different adaptation procedures with different organic solvents (e.g. toluene, 1-octanol) were tested and the resulting solvent tolerant phenotypes have been characterized and compared to non-solvent tolerant phenotypes. In a second step, genetic engineering was used to create knock-out mutants to overcome critical aspects of adapted solvent tolerant phenotypes such as poor reproducibility, tedious adaptation procedures, and low stability.

Biofilms of *Pseudomonas* sp. strain VLB120 have been cultivated in a specifically designed flow-cell and the influence of the solvent styrene was investigated. It became obvious, that although cells suffered severe damage upon the solvent shock, the biofilm organisms recovered and adapted to high concentrations of styrene [8]. Concomitantly the excretion of EPS was boosted upon the addition of this organic solvent.

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PSP033

The novel subtilase SprP influences the lifestyle of *Pseudomonas aeruginosa*

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P. aeruginosa is a very undemanding organism that is ubiquitously distributed. The bacterium can be found in wet or humid surroundings, ranging from soil to human and produces a huge variety of extracellular proteins. Hence, there exists a big potential for enzymes with suitable properties for biotechnological application. Several proteases belong to the arsenal of secreted enzymes. Some of these proteases like Elastase and Protease IV are well characterized but others exist of which nothing is known so far (Hoge *et al*., 2010). Proteases in general are highly relevant for technical enzyme applications. Subtilases for example are typical detergent proteases and are defined as serine proteases that belong to the peptidase_S8 family. These subtilases are encoded as preproenzymes carrying a signal peptide which drives their translocation through the cytoplasmic membrane and a propeptide acting as a folding mediator required to give the protease its final native conformation.

By homology, we have identified the open reading frame *PA1242* in the genome sequence of *P. aeruginosa* PAO1 encoding a so far hypothetical protein as a putative member of the E-H-S family of subtilases. The gene product of *PA1242* (*sprP*) contains a predicted signal sequence and a peptidase S8 domain. Sequence analysis revealed the presence of an additional element in the domain organization of the protease. SprP carries, beside its signal peptide and the S8 domain, a domain of unknown function (DUF) between both elements. After the identification of SprP, the gene was cloned, expressed in *E. coli* and the protease activity was measured with established protease substrates.

Often, proteases have an impact on different physiological processes like protein processing and activation, secretion of other proteins and pathogenicity of the host bacterium. A *P. aeruginosa sprP*-negative mutant was constructed and different phenotypes were tested to elucidate the physiological role of SprP.We were able to illustrate an eminent role of SprP by characterization of different phenotypes. Deletion of *sprP* causes an increased biofilm formation and pyoverdine biosynthesis, the accumulation of cell aggregates during growth, and a reduced growth under anaerobic conditions.

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PSP034

Will not be presented!

PSP035

The structure of the NADH: ubiquinone oxidoreductase from *Vibrio chloerae*

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Vibrio choleraemaintains a Na^+-gradient across the cytoplasmic membrane (1,2). The generated sodium motive force is essential for substrate uptake, motility, pathogenicity, or efflux of antibiotics. This gradient is generated by an NADH:ubiquinone oxidoreductase (NQR) that is related to the RNF complex of archea and bacteria. NQR is an integral membrane protein complex consisting of six different subunits, NqrA-NqrF (3,4). In order to get insights into the redox-driven Na^+-transport mechanism we have isolated and crystallized the NQR of *Vibrio cholerae* (5). The crystals of the entire membrane complex diffract so far to 3.7 Angstrom and provide first detailed structural information in this respiratory enzyme.

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PSP036

Proteome assessment of an organohalide respiring species: Dehalococcoides sp. CBDB1

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Chlorinated hydrocarbons that were released into the environment are due to their toxic and cancerogenic potential a threat to nature and human health. The ability of anaerobic bacteria belonging to *Dehalococcoides spp*. to use a broad range of chemicals from this class as terminal electron acceptors shows potential for bioremediation use. The strictly anaerobic *Dehalococcoides sp*. CBDB1 utilizes a wide range of electron acceptors with the help of its reductive dehalogenase enzymes. In the sequenced genome are 32 different reductive-dehalogenase-homologous (*rdh*) gene operons annotated [1]. The high number of *rdh* clusters sparks a special interest in the differences between the gene products. Strong substrate specificities of the encoded *rdh* genes can explain this. To analyse the

substrate specific expression of *rdh* genes, the proteome of bacteria cultivated under identical growth conditions but with different electron acceptors were analysed by using a liquid chromatography tandem mass spectrometric (LC-MS/MS) based approach with focus on the detected *rdh* proteins.
A simplified sample preparation procedure was used to achieve high protein coverage despite of low achievable cultivation densities and the small cell size of the bacteria. Harvested cells were lysed by mechanical treatment. After ultracentrifugation the membrane and cytosolic fraction were separately digested in solution using trypsin. Desalted peptides were directly used for LC-MS/MS measurements. Shotgun mass spectrometry resulted in an average of 660 identified proteins which is about 44% of all predicted gene products. Three dehalogenases were identified in all CBDB1 cultures in the presence of various halogenated hydrocarbons such as chlorinated aromatic and non-aromatic substances. Overall 14 different dehalogenases were detected giving first hints for different substrate-related expression patterns which were determined by label-free quantification. Exact quantification of *rdh* proteins is planned by selected reaction monitoring (SRM) mass spectrometry. Results from these experiments will further improve the understanding of the correlation between the electron acceptors provided during cultivation and expression of *rdh* enzymes by CBDB1.
Acknowledgement: This work is supported by the DFG (research unit FOR1530).
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PSP037
Identification of genes essential for anaerobic growth of *Listeria monocytogenes*

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The adaptation of *L. monocytogenes* to various growth conditions contributes to its ubiquitous distribution and its role as an important food borne pathogen. *L. monocytogenes* is resistant to many adverse environmental conditions, e.g. it growth at temperatures from 0 - 45 °C, in a pH range from pH 4.1 to 9.6 and at high salt concentrations. Furthermore, as a facultative anaerobic bacterium it can adapt to various oxygen tensions. Although early physiological studies on the anaerobic metabolism of *L. monocytogenes* have been completed with global approaches such as comparative genome-, transcriptome- and proteome analysis, aerobic growth of *L. monocytogenes* is still much better understood than anaerobic growth. In this study, the anaerobic growth of *L. monocytogenes* was further characterized.
We demonstrate that the transcriptional profile changes significantly in *L. monocytogenes* cells either grown aerobically or anaerobically in BHI medium at 37 °C. A set of 116 genes was stronger transcribed under aerobic conditions and a set of 26 genes was stronger transcribed under anaerobic conditions. For 19 of the 26 anaerobically stronger transcribed genes deletion or insertion mutants were constructed. The respective mutants are able to grow both aerobically and anaerobically, suggesting that their expression is not essential for anaerobic growth and proliferation of *L. monocytogenes*.
However, a high throughput screening of an insertion mutant banc (Joseph et al., 2006) identified genes essential for anaerobic growth. 11 out of 1360 investigated insertion mutants showed an anaerobic sensitive phenotype while they were able to grow aerobically. Interestingly, all these mutants are interrupted in the *atp*-locus, which showed no differential transcription dependent on the oxygen availability. The essential function of the *atp*-locus for anaerobic growth was further validated by growth analysis of the deletion mutants *L. monocytogenes*/ΔatpA, *L. monocytogenes*/ΔatpB and *L. monocytogenes*/ΔatpD and subsequent complementation of the deleted genes. These results indicate that the expression of a functional F_0F_1-ATPase is essential for growth and proliferation during anaerobic but not during aerobic growth in *L. monocytogenes*.

PSP038
Biochemistry of Ethylbenzene Dehydrogenase, the key enzyme of the anaerobic ethylbenzene degradation in *Azoarcus sp.* strain EbN1

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The initial reaction of the anaerobic degradation pathway of ethylbenzene in *Azoarcus sp*. strain EbN1 ("*Aromatoleum aromaticum*") is an oxygen-independent and stereospecific hydroxylation of ethylbenzene to (S)-1-phenylethanol by the molybdenum/iron sulfur/heme enzyme ethylbenzene dehydrogenase (EbDH, 1). EbDH is a heterotrimer of 3 subunits with a total molecular mass of 160 kDa and belongs to the DMSO reductase family of molybdenum enzymes (Type II). The α subunit (96 kDa) carries a *bis*-molybdopterin cofactor, which is the active site of the enzyme, and a FeS cluster. The β subunit (43 kDa) carries 4 FeS clusters, which are responsible for the electron transport from the active site of the enzyme to thehemeb cofactor in the γ subunit (23 kDa; 2). In the last years, comparison of kinetic data of ethylbenzene analogs which act as EbDH substrates as well as chromatographic analysis of the formed alcohols lead to a first model of the catalytic mechanism of the enzyme (3). Furthermore, quantum chemical calculations of the EbDH reaction mechanism were performed and supported the kinetic and chromatographic data (4). Recently, new ethylbenzene analogs were tested as possible new substrates or inhibitors of the enzyme. The kinetic data of these new compounds together with chromatographic data of the formed alcohol products reveal new insights into the catalytic mechanism and the enantioselectivity of the enzyme. In addition, specifically deuterated ethylbenzene derivates where synthesized to test which of the hydrogen atoms on the C1 position of the ethyl group of ethylbenzene is abstracted during catalysis and replaced by a water-derived hydroxyl group. Kinetic investigations and mass spectrometric analysis of the products indicate that the proS hydrogen is abstracted during enzymatic catalysis.
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PSP039
Norepinephrine and epinephrine stimulate growth and motility of *Vibrio cholerae*

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Under stress the body produces biochemical messengers like catecholamine hormones to adapt to the specific situation. Attenuation of the immune response by stress hormones, together with stimulation of bacterial growth due to hormone exposure, leads to increased susceptibility of the host to bacterial infections.
A stimulatory effect of norepinephrine (NE) and epinephrine (Epi) on growth ofSalmonellaserovartyphimuriumwas observed in SAPI medium which contains serum proteins including the Fe-binding protein transferrin (Pullinger et al. 2010). Under these growth conditions, NE competes with transferrin for Fe by complexation of Fe with its catechol moiety. Hereby, the availability of this important trace element increases, which in turn stimulates growth of the pathogen. NE and Epi were also shown to promote swarming ofS. typhimuriumon plates consisting of LB broth with 0.3% agar (Moreira et al. 2010). We investigated the effect of NE or Epi on the growth ofVibrio choleraestrain O395-N1 in SAPI-serum medium. No growth ofV. choleraestrain RIMD2203102 was observed on SAPI-serum medium with added NE or Epi (Nakano et al. 2007). WithV. choleraestrain O139-N1 grown under similar conditions, NE led to a two-fold increase in growth yield after 46 h. Both catecholamines stimulated motility of strain O139-N1 on SAPI-serum swarming plates, but inhibited swarming on plates consisting of minimal medium with glucose as carbon source. We propose that in the presence of serum proteins, the iron limitation caused by transferrin was overcome by the catecholamines, resulting in increased motility ofV. choleraeO139-N1 compared to the hormone-free control. We suggest that in minimal medium, the iron-chelating properties of NE and Epi led to a decrease in free iron, and resulted in diminished motility ofV. choleraecells when compared to cells exposed to swarming plates devoid of the hormones.
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PSP040
Three different principles of ketone carboxylation in *Aromatoleum aromaticum*

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The b-proteobacterium *Aromatoleum aromaticum* degrades aliphatic and aromatic ketones (e.g. acetone, butanone, acetophenone, 4-hydroxyacetophenone) as single substrates under aerobic and denitrifying conditions. Degradation of each substrate is initiated by a carboxylation reaction catalysed by specific, substrate-induced carboxylases.
Acetone carboxylase (Acx) carboxylates acetone and butanone in an ATP-dependent, biotin-independent reaction. 2 ATP are hydrolyzed to 2 AMP and 4 Pi for one acetone carboxylated. Acx is present in cells grown aerobically or anaerobically on acetone or butanone, but not in cells grown

on acetophenone or acetate. It consists of 3 subunits (85, 75, and 20 kDa) in an $(\alpha\beta\gamma)_2$ composition and contains 2 Fe and 1 Zn per native complex. Interestingly, known acetone carboxylases from other organisms (e.g. *Paracoccus denitrificans*, *Rhodobacter capsulatus*, *Geobacillus thermoglucosidasius*) differ in in metal content and ATP hydrolysis stoichiometry, despite their high sequence similarities.
Acetophenone carboxylase (Apc) is present in cells grown aerobically or anaerobically on acetophenone, but not in cells grown on acetone or 4-hydroxyacetophenone. Acetophenone is carboxylated to benzoylacetate concomitant with hydrolysis of 2 ATP to 2 ADP and 2 Pi. 4-hydroxyacetophenone is not a substrate of Apc. The enzyme consists of 5 subunits (87, 75, 70,34, and 15 kDa) in an $(\alpha\beta\beta'\gamma)_2\varepsilon_2$ composition. Four of the five subunits show high sequence similarity to the subunits of Acx, while the ε-subunit is unique. 2 Zn per native complex were identified as cofactors, but no Fe or biotin. The observed reaction mechanisms of acetone carboxylase and acetophenone carboxylase represent novel ATP-dependent, biotin-independent carboxylation mechanisms in bacterial ketone catabolism, which likely involve the transient activation of both substrates via phosphorylation.
4-Hydroxyacetophenone carboxylase (Xcc) belongs to the class of biotin-dependent carboxylases and consists of 3 subunits: a biotin carboxyl carrier protein (18 kDa) and 2 carboxylase subunits (50, 55 kDa). Therefore, despite the similarity of the respective substrates, completely different carboxylation mechanisms are employed for the carboxylation of acetophenone and 4-hydroxyacetophenone.

PSP041
Biosynthesis and attachment of open-chain tetrapyrroles in cryptophytes

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Phycobiliproteins are light-harvesting proteins, which occur in cyanobacteria, red algae and cryptophytes in addition to chlorophyll containing antenna complexes. They allow the organisms to efficiently absorb light in regions of the visible spectrum that are poorly covered by chlorophylls. Cryptophytes are unicellular, eukaryotic algae and widespread in marine and limnic waters. Their phycobiliproteins consist of an (αα'ββ) heterotetrameric apo-protein covalently associated with characteristic open chain tetrapyrroles, which act as light absorbing chromophores. Cryptophytes employ the six different chromophores phycocyanobilin (PCB), phycoerythrobilin (PEB), 15,16-dihydrobiliverdin (15,16-DHBV), mesobiliverdin (MBV), bilin 584 and bilin 618 for light-harvesting.
The biosynthetic pathway of open chain tetrapyrroles in cryptophytes is entirely unknown. The model organismGuillardia thetauses the phycobiliprotein PE545, which is associated with the chromophores 15,16-DHBV and PEB. This is an interesting fact, because 15,16-DHBV occurs only as an intermediate of PEB biosynthesis in cyanobacteria and cyanophages but not as a bound chromophore. This raises the question of elucidating the chromophore biosynthesis and attachment in the cryptophyteG. theta. Extensive bioinformatic analyses and amino acid sequence alignments identified a putative heme oxygenase, two putative bilin reductases and different putative phycobiliprotein lyases inG. theta.
Currently, the enzymatic activities of these putative bilin biosynthesis enzymes are analyzed. First results give some indications that the heme oxygenase is able to cleave heme yielding the open-chain tetrapyrrole biliverdin IXα. Furthermore a bilin reductase reducing 15,16-DHBV to PEB could be identified, which will be further investigated via crystallization studies. The enzymatic activity of a second bilin reductase will also be examined as well as the attachment of the PEB molecules to the PE545-β subunits and especially the 15,16 DHBV molecules to the PE545-α subunits.

PSP042
Itaconate degradation may be important for pathogenesis

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Itaconate (methylenesuccinate) has recently been shown as a mammalian metabolite whose production is induced during macrophage activation (1). This compound is a potent inhibitor of isocitrate lyase (2), which is important for survival of many pathogens inside the host (3). We have shown that numerous pathogens includingYersinia pestisandPseudomonas aeruginosapossess genes for itaconate degradation, which were previously shown as pathogenesis-related in some species (4,5). Furthermore, we heterologously overproduced and characterized in detail a key enzyme of the itaconate degradation pathway, (S)-citramalyl-CoA lyase, fromY. pestisandP. aeruginosa. Besides bacteria, this enzyme is present in mammals. Interestingly, the corresponding gene was previously shown to be highly expressed in some tumor cell lines with high metastatic potential(6). Itaconate detoxification might be important for these cells, since this compound is an indirect inhibitor of phosphofructokinase (7) and therefore of the glycolysis, the main bioenergetic process in tumor cells. Thus, itaconate degradation pathway may be considered as a perspective target for the development of novel therapeutic agents.

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PSP043
Cac0116 of *Clostridium acetobutylicum* - a carbon monoxide dehydrogenase?

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Carbon monoxide dehydrogenases (CODHs) of anaerobic Organisms are enzymes with a special nickel, iron and sulphur containing cluster, enabling the reversible oxidation of CO to CO_2 [1]. CODHs are involved in several metabolic functions like energy conservation, autotrophic CO_2-fixation or reductive regeneration of NADPH. Another function was postulated for CODH-IV of the hydrogenogenic bacterium *Carboxydothermus hydrogenoformans*. Based on the fact that the CODH-IV gene is located in a cluster of genes that might be necessary for the detoxification of reactive oxygen species (ROS), a hydrogen peroxide reducing role was discussed [2].
In *Clostridium acetobutylicum* the genes *cac0116* and *cac2498* are annotated as CODHs. Recent studies demonstrated a highly upregulation (24 fold) of the gene *cac0116* under oxidative stress leading to the conclusion that its gene product is part of the ROS detoxification system [3].
Here, we report on the purification of Cac0116 after overexpression in *E. coli* and *C. acetobutylicum*. So far, our results did not reveal any CODH activity of this enzyme. Furthermore, a specific *cac0116* knock out mutant was constructed by using the ClosTron® technology [4]. Comparative characterisation of the phenotypes (optical density, pH, product spectrum, produced gases) of the knock out mutant, the overexpression strain and the wild type strain of *C. acetobutylicum* indicated in the knock out mutant a reduced glucose consumption. Interestingly, the H_2:CO_2 ratio seemed to be altered, when the *cac0116* gene was inactivated. This suggests a function of Cac0116 in electron transfer processes, directly or indirectly coupled with H_2 production in *C. acetobutylicum*.

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PSP044
Sulfur metabolism in the thermoacidophilic archaeon *Metallosphaera cuprina*: insights from genome analysis and gene expression studies

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The thermoacidophilic archaeon *Metallosphaera cuprina* Ar-4, originally isolated from a sulfuric hot spring, Tengchong, Yunnan, China, has the ability to oxidize reduced inorganic sulfur compounds (RISC) [1]. The genome has been completely sequenced and annotated. It consists of a 1,840,348 bp circular chromosome (2029 ORFs) [2], including at least 35 genes putatively related to sulfur metabolism.
Genes potentially encoding a heterodisulfide reductase complex HdrABC are found in several archaeal and bacterial sulfur oxidizers. Corresponding genes also exist in *Metallospahera cuprina* and are part of a gene cluster (*mcup*_0681-0689) that also comprises *dsrE* and *sirA* like genes. In bacteria, rhodanese (thiosulfate:cyanide sulfurtransferase) encoding genes often occur in immediate vicinity of *dsrE-sirA* homologous genes. Proteins of the DsrE and SirA families have been implicated to be involved in sulfur transfer reactions not only during biosynthesis of sulfur-containing cell constitutents like thiouridine [3] but also during oxidative sulfur metabolism [4]. In both, the archaeon *Metallosphaera sedula* [5] and the proteobacterium *Acidithiobacillus ferrooxidans* [6], the *hdr* gene cluster including the *sirA* and *dsrE* homologs is highly upregulated by RISC further stressing a potential prominent role of the encoded proteins in oxidative sulfur metabolism.
Mcup_0681 and Mcup_0682 from *M. cuprina* share 26% identity and both possess characteristic features of DsrE family proteins. Mcup_0683 is assigned as a SirA family protein. Mcup_0681-0683 were overproduced in *E. coli*. Both, Mcup_0681 and Mcup_0682, were identified as homotrimers by gel permeation chromatography while Mcup_0683 is a monomer. Strong and specific interaction between Mcup_0681 and Mcup_0683 was detected by co-chromatography of pairs of tagged and untagged proteins on *Strep*-Tactin columns. All three proteins contain a strictly conserved cysteine residue in a

potential active site region. We demonstrated the ability of Mcup_0683 to bind sulfur via MALDI-TOF mass spectrometry. These findings provide strong motivation to investigate the potential of proteins Mcup_0681-0683 to act together in a sulfur relay system involved in dissimilatory sulfur oxidation in *Metallosphaera cuprina* and possibly also in other archaea and bacteria.

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PSP045

Elucidation of the Periplasmic Cytochrome Network in *Shewanella oneidensis* MR-1

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Shewanella oneidensis MR-1 is a Gram-negative soil bacterium which shows an astonishing versatility in terms of electron acceptors it can use. The predominant proteins driving respiratory electron transfer from the cytoplasm to periplasmic space and from there to the outer membrane are *c*-type cytochromes. Interestingly, *S. oneidensis* cells express a large number of periplasmic *c*-type cytochromes that are not primarily involved in iron reduction (i.e. SoxA-like, NrfA and CcpA) even when they grow under iron reducing conditions. Furthermore, our experiments revealed that iron grown cells are able to conduct electron transfer to a multitude of electron acceptors although they had not been in contact to one of these acceptors in the growth medium. It seems fairly possible that these periplasmic *c*-type cytochromes build up a network which allows electron exchange between respiratory pathways. This feature would certainly enable the cell to quickly respond to changes in the availability of electron acceptors that occur in its environment. Examples for connected respiratory pathways will be presented. Still, although it is generally believed that *c*-type cytochromes conduct rather unspecific electron transfer it was possible to show that is not necessarily the case. The electron transport pathway to the peroxidase CcpA is an example for specificity within *c*-type cytochrome dependent electron transfer. The two cytochromes involved, CcpA and ScyA, are disconnected from other pathways. CcpA functions as a peroxidase protecting the cell against oxidative stress caused by hydrogen peroxide possibly produced during dissimilatory iron reduction via the Fenton reaction. CcpA gains its electrons exclusively from ScyA, a small monoheme cytochrome. In this study the range and dynamic of the periplasmic *c*-type cytochrome network will be presented in further detail.

PSP046

Complete β-oxidation of the acyl side chain of cholate by *Pseudomonas* sp. strain Chol1 *in vitro*

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Steroids are ubiquitous natural compounds with diverse functions in eukaryotes. In bacteria, steroids occur only as rare exceptions but the ability of transforming and degrading steroids is widespread among bacteria.
We investigate bacterial steroid degradation using the bile salt cholate as a model compound and *Pseudomonas* sp. strain Chol1 as a model organism. Cholate degradation is initiated by oxidative reactions at the A-ring followed by cleavage of the side chain attached to C17. Mutants of strain Chol1 with defects in the genes *skt* and *acad* are defect in the degradation of the acyl side chain. In culture supernatants of these mutants, (22E)-7α,12α-dihydroxy-3-oxochola-1,4,22-triene-24-oate (DHOCTO) and 7α,12α-dihydroxy-3-oxopregna-1,4-diene-20-carboxylate (DHOPDC), respectively, accumulate as dead end products. The structure of these compounds indicates that degradation of the acyl side chain proceeds via β-oxidation but explicit *in vitro* data was missing so far. We investigated the degradation of the acyl side *in vitro* using cell extracts of strain Chol1 in the presence of co-factors (CoA, ATP, NAD^+ and phenazinemetholsulfate). When cholate or $\Delta^{1,4}$-3-ketocholate were used as substrates, $\Delta^{1,4}$-3-ketocholyl-CoA was the end product indicating that further oxidation of the acyl side chain was not possible *in vitro* under the applied conditions. When either DHOCTO or DHOPDC were used the complete side chain was cleaved off *in vitro* leading to 7α,12α-dihydroxy-androsta-1,4-diene-3,17-dione (12α-DHADD) as end product. With both substrates the CoA-ester of DHOPDC accumulated transiently in the assay.
With cell extracts of the *skt* mutant DHOCTO was converted to DHOCTO-CoA which was not further degraded to 12α-DHADD. With cell extracts of the *acad* mutant DHOCTO was converted to DHOPDC-CoA, which was also not further degraded. Thus, the phenotypes of both mutants were confirmed by these *in vitro* assays.
To our knowledge this is the first detailed *in vitro* demonstration of the complete degradation of a steroid side chain by β-oxidation in bacteria. Furthermore, our results indicate that the dehydrogenation reactions of $\Delta^{1,4}$-3-ketocholyl-CoA and of DHOPDC-CoA are the rate limiting steps in this β-oxidation pathway.

PSP047

Genomic plasticity responsible for dissimilatory iron reduction in *Shewanella oneidensis* MR-1

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The ability of the facultative anaerobic bacterium *Shewanella oneidensis* MR-1 to respire poorly soluble electron acceptors under anoxic conditions relies on a complex electron transfer network. Four distinct pathways predicted to facilitate respiratory electron flow to extracellular electron acceptors are encoded in the genome of *S. oneidensis* MR-1. These pathways share MtrA (metalreducing protein A) and MtrB paralogues, which are periplasmic c-type chytochromes and integral outer membrane beta-barrel proteins, respectively (1). Interestingly gene clusters encoding MtrA and MtrB homologs are phylogenetically distributed among all classes of proteobacteria and the corresponding proteins were shown to be not only involved in ferric iron reduction but also ferrous iron oxidation (2).
A *mtrB* null mutant strain in *Shewanella* lacks the ability to respire on Fe(III)-oxides (3). Interstingly, after prolonged incubation supressor mutations occur that rescue the mutant phenotype. In this work we isolated and characterized such a *ΔmtrB* suppressor mutant. Molecular and genetic analysis revealed that the suppression relies on a functional replacement of MtrB and MtrA by homologous proteins encoded by SO4359 and SO4360 respectively. This replacement underlies a transcriptional upregulation of the SO4362-SO4357 gene cluster which was found to be due to an insertion sequence (ISSod1) belonging to the IS-1 superfamily generating a constitutively active hybrid promoter. Here we could show for the first time a functional replacement of the MtrAB subcomplex by a complex consistent of homologous proteins and the involvment of SO4360 as periplasmic electron carrier in dissimilatory iron reduction in *Shewanella oneidensis* MR-1.

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PSP048

The phosphotransferase system CAC0231-CAC0234 controls fructose utilization of *Clostridium acetobutylicum*

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Clostridium acetobutylicum is well characterized by its biphasic fermentation metabolism. At higher pH values exponentially growing cells usually produce acetate and butyrate as main fermentation products whereas when the pH has dropped below 5.0 the metabolism switches to 'solventogenesis' with the dominating fermentation products butanol and acetone. As a carbon and energy source a variety of carbohydrates like glucose, fructose or xylose can be utilized by *C. acetobutylicum*.
Generally, carbohydrates were taken up via three types of transporters: symporter, ATP-binding cassette (ABC) transporter and phosphotransferase systems (PTS). For the uptake of hexoses, hexitols and disaccharides thirteen PTS have been identified in *C. acetobutylicum*. Among them, three PTS are supposed to be responsible for the uptake of fructose. The apparent primary fructose transport system is encoded by a polycistronic operon (*cac0231-cac0234*) including a putative DeoR-type transcriptional regulator (CAC0231), a 1-phosphofructokinase (CAC0232), a PTS IIA (CAC0233) and a PTS IIBC (CAC0234). To analyze the role of the PTS during growth on fructose as sole carbon source, each single gene of the operon (*cac0231-cac0234*) was specifically interrupted using the ClosTron® system. All mutant strains showed impaired growth due to reduced fructose consumption. Interestingly, a concomitant loss of solvent production was monitored indicating a threshold of sugar concentration for initiation of the metabolic switch.
Moreover, the transcriptional regulator CAC0231 was overexpressed in *E. coli* and purified for electrophoretic mobility shift assays (EMSA). Here, a putative binding motif was identified and proved by a specific binding of CAC0231 to the promoter region of *cac0231-cac0234*.

PSP049

Characterization of plasmid pPO1 from the hyperacidophile *Picrophilus oshimae*

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Picrophilus oshimae and *Picrophilus torridus* are free-living, moderately thermophilic and acidophilic organisms from the lineage of *Euryarchaeota*. With a pH optimum of growth at pH 0.7 and the ability to even withstand molar concentrations of sulphuric acid, these organisms represent the most extreme acidophiles known. So far, nothing is known about plasmid biology in these hyperacidophiles. Also, there are no genetic tools available for this genus. We have mobilized the 7.6 Kbp plasmid from *P. oshimae* in *E. coli* by introducing origin-containing transposons and describe the plasmid in terms of its nucleotide sequence, copy number in the native host, mode of replication and transcriptional start sites of the encoded ORFs. Plasmid pPO1 may encode a restriction/modification system in addition to its replication functions. The information gained from the pPO1 plasmid may prove useful in developing a cloning system for this group of extreme acidophiles.

PSP050

The three NiFe-hydrogenases of *Sulfurospirillum multivorans*: Insights into the hydrogen metabolism of an organohalide respiring bacterium

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One of the simplest reactions in nature, the oxidation of molecular hydrogen and its reverse reaction, is catalysed by a group of enzymes called hydrogenases. Opposed to the simplicity of this reaction, hydrogenases are complex enzymes with several metal-containing cofactors. They appear in multifaceted forms, often in one single organism, where they fulfill different physiological roles. One of the largest groups of hydrogenases harbour one nickel and one iron atom in their catalytic center. Thus, they are called NiFe-hydrogenases.
Sulfurospirillum multivorans, an organohalide-respiring ε-proteobacterium, harbours the genes coding for at least three NiFe-hydrogenases, none of them hitherto investigated. The most prominent role of energy conservation via the oxidation of H_2 is fulfilled presumably by a membrane-bound uptake hydrogenase, similar to the MBH of *Wolinella succinogenes*. The same gene cluster comprises a second hydrogenase, whose physiological role is unclear. It is similar to hydrogenase 3 from *Aquifex aeolicus* and, to a lesser extent to regulatory hydrogenases and cyanobacterial uptake hydrogenases. The third hydrogenase, encoded by four genes spatially separated from the other hydrogenase gene cluster, is related to H_2-evolving energy converting hydrogenases (Ech) and might act as an electron sink, as we have detected H_2 production during microaerobic growth after depletion of oxygen. Remarkably, the membrane subunits normally present in these hydrogenases, referring to a proton pump and an electron-transferring subunit of complex I, are missing on the according *S. multivorans* gene cluster. This raises the question, whether the enzyme binds to the according proteins of the respiratory chain present in the organism, or if it resides freely in the cytoplasm. In order to understand the physiological role of these so far under-characterised NiFe-hydrogenases in *S. multivorans*, growth experiments, transcription analysis and subcellular localisation studies accompanied by activity measurements were carried out, whereas purification, spectroscopical analyses and genetic modifications are planned.
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PSP051

Analysis of the dual flagellar stator system in *Shewanella oneidensis* MR-1 at the single-cell level

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Flagella are rotating filaments driven by a motor complex at the filaments base which is powered by the sodium- or proton-motive force. The motor consists of two major structures, the rotating switch complex and the stator complexes that surround the rotor in a ring-like fashion. The stator complexes within this stator ring system are constantly exchanged with a membrane-located pool of precomplexes that are activated upon incorporation into the motor.
Recent studies on the gammproteobacterium *Shewanella oneidensis* MR-1 revealed that two different sets of stators, annotated as PomAB and MotAB, differentially support the rotation of a single polar flagellum. PomAB, the dominant stator complex, is sodium-ion dependent, and MotAB, most likely acquired by lateral gene transfer, is proton dependent. Physiological and localisation studies provide evidence that the rotor-stator configuration in the flagellar motor is adjusted to environmental sodium-ion concentrations through an exchange of stator complexes. Both stators appear to be simultaneously incorporated into the flagellar motor under low sodium-ion concentrations, suggesting that *S. oneidensis* MR-1 has a hybrid motor that concurrently uses sodium-ions and protons. A global database analysis of bacterial genomes revealed that dual or multiple stator systems are surprisingly common among bacteria. To demonstrate, for the first time, the existence of a naturally occuring flagellar hybrid motor, flagellar performance was analysed at the single cell level. To this end, 'tethered cell' and 'bead'-assays were established. Using these assays in concert with fluorescent microscopy on labeled stator components, we performed *in vivo* analysis of the stator ring composition and dynamics. The results give insights into the dynamic adaption of the flagellar motor configuration in dependence of the environmental sodium-ion concentrations.

PSP052

Activity and localization of Dehydrogenases in *Gluconobacter oxydans*

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Bacterial cytoplasmic and intracytoplasmic membranes are important mounting plates for all types of proteins directly or indirectly involved in electron transport and generation of proton gradients. In *Gluconobacter oxydans* membrane-bound dehydrogenases are exposed to the periplasm and funnel reducing equivalents from educts to the electron transport chain, thereby releasing diverse incompletely oxidized products. Here, we analyze the activities and expression of membrane-bound dehydrogenases of *Gluconobacter oxydans* under different growth conditions with ethanol, glucose, glycerol, mannitol and sorbitol as substrates. Osmotic stress and oxygen partial pressure increases specific activities by up to one order of magnitude. Measurements of enzyme activities were also supported by immunolocalization of two key enzymes, the PQQ-dependant membrane-bound sorbitol dehydrogenase and the quinol oxidase in *Gluconobacter* cells. This technique allows a semi-quantitative estimation of enzyme expression and, at the same time, localization at subcellular level.

PSP053

Alternative fructose utilization in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is used for the industrial scale production of amino acids, such as the feed additive L-lysine or the flavor enhancer L-glutamate. Predominantly the fermentation of amino acids is carried out using sugar substrates, such as glucose, sucrose, and fructose, which are all substrates of the phosphotransferase system (PTS) in *C. glutamicum*. The utilization of fructose starts by PTS mediated uptake and simultaneous phosphoenolpyruvate dependent phosphorylation to fructose-1-phosphate. Subsequently fructose-1-phosphate is phosphorylated to the glycolytic intermediate fructose-1,6-bisphosphate by 1-phosphofructokinases.
To analyze the role of the 1-phosphofructokinases in *C. glutamicum* deletion mutants of the corresponding genes *fruK1* and/or *fruK2* were constructed. The presence of one of the 1-phosphofructokinase genes was sufficient for growth with fructose whereas *fruK1* encoded the more important 1-phosphofructokinase as only Δ*fruK1* and not Δ*fruK2* showed impaired growth compared to the WT. Growth with fructose was completely inhibited when both genes *fruK1* and *fruK2* were deleted (Δ*fruK1*Δ*fruK2*).
Suppressor mutants were isolated after prolonged incubation of Δ*fruK1*Δ*fruK2* in fructose minimal medium. These suppressor mutants regained the ability to grow from fructose. Growth rates of the suppressor mutants were comparable to the WT with a concomittant increase of biomass yields of the suppressor mutants. The biomass increase is likely due to the reduced acid byproduct formation. When testing for L-lysine production from fructose, the suppressor mutants showed strong increased L-lysine production compared to the parental strain.

PSP054

Elucidation of the tetrachloroethene respiratory chain in *Sulfurospirillum multivorans*

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The composition of the electron transfer chain leading from the oxidation of formate (or hydrogen) to the reduction of tetrachloroethene (PCE) in the inner membrane of the gram-negative epsilonproteobacterium *S. multivorans*i s an enigma unsolved to date. Under anoxic conditions the organism is able to couple the reductive dechlorination of PCE to ATP synthesis via electron transport phosphorylation (organohalide respiration). The terminal reductase, the PCE dehalogenase (PceA), is a corrinoid-containing iron-sulfur protein. The *pceA* gene is accompanied by a small gene *pceB* that encodes a putative membrane anchor of the enzyme. In cells grown with PCE, PceA is located on the periplasmic face of the cytoplasmic membrane [1]. In cells cultivated with fumarate instead of PCE the enzyme is almost exclusively located in the cytoplasm.
In this study differentially pre-conditioned cells (or membrane vesicles thereof) with different PceA localization patterns were treated with reduced quinone analoga (e. g. Plumbagin) to test for the involvement of quinones in the electron transfer. With 2-heptyl-4-hydroxy quinoline-N-oxide (HQNO) the inhibition of quinone-dependent electron shuttling was tested. The previously proposed necessity of a proton gradient for the reductive dechlorination [2] was proven by the use of uncouplers (e. g. CCCP). The combination of genomic data and results from differential proteome analysis (together with Seifert, J. and von Bergen, M.; UFZ Leipzig) allowed for the identification of membrane-associated proteins only found in membrane preparations of PCE-grown cells (e. g. quinol dehydrogenase). Experiments are underway to purify and characterize these putative components of the electron transfer pathway. The expression of the respective genes in PCE- or fumarate-grown cells was tested. From the results presented here an actual model of the PCE respiratory chain in *S. multivorans* is derived and will be discussed.

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PSP055

Quantitative determination of *in vivo* ATP concentrations in *Corynebacterium glutamicum* using fluorescent indicator proteins

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Corynebacterium glutamicum is a Gram-positive, non-pathogenic soil bacterium applied in the large scale industrial production of amino acids. Additionally it serves as model organism within the suborder *Corynebacterianeae*, which also comprises pathogenic strains like *Corynebacterium ulcerans* and *Mycobacterium tuberculosis*, which possess a partially intracellular life style.
For the analysis of intracellular metabolite concentrations in bacteria mainly disruptive methods have been applied in the past. Thereby restricted temporal resolution, dilution of the sample and contamination of samples by extracellular solutes can be major disadvantages. Furthermore, these methods are difficult to apply for the metabolite analysis of intracellular pathogens [1].
Recently genetically encoded nanosensors for different metabolites like maltose, glucose, and sucrose have been developed, which enable the determination of metabolite levels by optical means in a non-disruptive manner [2]. These sensors are composed of a binding protein terminally fused to two different spectral variants of GFP like CFP and YFP. They translate the binding of a metabolite into a change of Förster-Resonance-Energy-Transfer (FRET) efficiency between the two fluorescent proteins and thus allow quantification of metabolites.
We here adopt a series of genetically encoded ATP nanosensors developed by Imamura *et al*. [3] for the utilisation in *C. glutamicum* and related *Corynebacterianeae*. Therefore we cloned the genes encoding the ATP sensors with different binding affinities into suitable plasmids for their expression in *C. glutamicum* and related species. To determine *in vivo* concentrations of ATP a novel technique for calibration of the sensors in permeabilised cells was developed. Using these *in vivo* calibration curves as a reference, we analyzed intracellular ATP concentrations in *C. glutamicum* in the course of cultivation, which corresponded well with concentrations determined enzymatically.
In conclusion, we here show that genetically encoded nanosensors are a promising alternative for classical metabolite analysis avoiding severe restrictions within the application of the latter.

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PSP056

Effect of temperature on nitrogen metabolism in *Listeria monocytogenes*

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The human pathogen *L. monocytogenes* is frequently found in the environment. Therefore, this bacterium has to adapt to various conditions, depending on its actual ecological niche. Besides different environmental temperatures, for example 37 ºC within the host and 0 - 30 ºC in the environment, the availability of nutrients is another important factor the bacteria have to deal with. In the present work, the global transcriptome of *L. monocytogenes* EGDe grown in a defined medium with glucose as sole carbon source and glutamine as nitrogen source was analyzed and compared at 37 ºC and 24 ºC, using microarray technology. Microarray data were verified via qRT-PCR. At 24 °C the transcription level of the genes lmo1298 (encoding for GlnR, the glutamine synthetase repressor), lmo1299 (encoding for GlnA, the glutamine synthetase), lmo1516 (encoding AmtB, an ammonium transporter) and lmo1517 (encoding a protein similar to nitrogen regulatory PII protein) was much higher than at 37 °C. This observation is quite interesting in particular as no ammonium was added to the defined growth medium. A higher transcription of homologous genes in *B. subtilis* indicates nitrogen, more precisely glutamine-starvation. These data suggest that the glutamine-supply of *L. monocytogenes* is better at 37 °C compared to 24 °C. Growth analysis of *L. monocytogenes* in defined medium with glutamine or ammonia as nitrogen source at different temperatures support this assumption. Further studies will be carried out to characterize this temperature dependent transcriptional regulation of genes involved in nitrogen metabolism.

PSP057

Heterologous production of a bacterial Na^+ F_1F_o ATP synthase with a F_oV_o hybrid motor

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The Na^+ F_1F_o ATP synthase of the anaerobic, acetogenic bacterium *Acetobacterium woodii* is unique because it has a F_oV_o hybrid motor made by an 8 kDa bacterial F_o-like *c* subunit with two transmembrane helices, and an 18 kDa eukaryal V_o-like *c* subunit with four transmembrane helices but only one binding site. The stoichiometry of the subunits in the *c* ring as determined by laser-induced liquid beam ion desorption was 9 : 1 (F_o:V_o) (1). To begin a molecular study, we cloned the entire *atp* operon into the expression vector pTrc99A (Amersham Bioscience), introduced a His_6-tag at the N-terminus of the β subunit and transformed the resulting plasmid into the ATP synthase negative *Escherichia coli* strain DK8. The heterologously produced ATP synthase was purified in one step by Ni^{2+}-NTA affinity chromatography. The presence of all subunits was determined by peptide mass fingerprinting and by Western blots. The ATP synthase was functionally coupled which was shown by inhibition of ATP hydrolysis by DCCD. ATPase activity was stimulated by Na^+ and accompanied by Na^+ transport into proteoliposomes. The F_oV_o hybrid motor was purified with the established protocol for the purification of the *c* ring. Both types of *c* subunits were present in the *c* ring. The stoichiometry was determined by laser-induced liquid beam ion desorption with 9 : 1 (F_o:V_o).
From our results we can conclude that the heterologously produced Na^+ F_1F_o ATP synthase is functionally coupled and that *E. coli* is able to assemble the special F_oV_o hybrid motor. With this system we can now start with mutational analysis of the *c* subunits to get a better understanding of the physiological function of the hybrid motor.

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PSP058

Functional specificity of extracellular nucleases in *Shewanella oneidensis* MR-1

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Bacterial species such as *Shewanella oneidensis* MR-1 require extracellular nucleolytic activity for utilizing extracellular DNA (eDNA) as source of nutrients and for turnover of eDNA as structural matrix component during biofilm formation. We have previously characterized two extracellular nucleases in *S. oneidensis* MR-1, ExeM and ExeS. Although both are involved in biofilm formation, they are not specifically required to utilize eDNA as nutrient. Here we identified and characterized EndA, a third extracellular nuclease in *Shewanella*. The heterologously overproduced and purified protein was highly active and rapidly degraded linear and supercoiled DNA of various origins. Divalent metal ions Mg^{2+}

or Mn^{2+} were required for function. *endA* is co-transcribed with an extracellular phosphatase *phoA* and not upregulated upon phosphostarvation. Deletion of *endA* abolished extracellular degradation of DNA by *S. oneidensis* MR-1 and the ability to use eDNA as sole source of phosphorus. PhoA is not strictly required to exploit eDNA as nutrient. The activity of EndA prevents the formation of large cell aggregates during planktonic growth. However, in contrast to ExeM a deletion of *endA* had only minor effects on biofilm formation. The findings underline the importance of extracellular nucleolytic activity for *Shewanella* and strongly suggest specific functions for the different nucleases.

PSP059

Caffeate respiration in the acetogenic bacterium *Acetobacterium woodii*: Characterization of a caffeate-activating CoA transferase

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The anaerobic acetogenic bacterium *Acetobacterium woodii* couples the reduction of caffeate with electrons derived from molecular hydrogen to the synthesis of ATP by a chemiosmotic mechanism with Na^+ as coupling ions. This process is called caffeate respiration (1). The Na^+-translocating enzyme in this respiratory pathway was identified as a ferredoxin:NAD^+-oxidoreductase (Rnf) (2,3). Recently, the enzymes involved in caffeate reduction with electrons derived from the Rnf complex could be shown to be encoded by the so called caffeate reduction operon *carABCDE* (4). The first gene of the operon, *carA*, was annotated as a putative CoA transferase. To further elucidate the function of CarA, the gene was cloned into pET21a, heterologously overproduced in *Escherichia coli* and purified to apparent homogenity *via* IMAC. In a photometric assay, CarA of *A. woodii* could be affirmed as a hydrocaffeyl-CoA:caffeate CoA transferase. The biochemical properties of the enzyme are described and its role in the caffeate reducing process is discussed.

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PSP060

A bacterial electron bifurcating uptake hydrogenase

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A [FeFe]-hydrogenase containing four subunits (HydABCD) was purified from the cytoplasm of *Acetobacterium woodii* and the encoding genes were identified. The complex is predicted to have one [H]-cluster, three [2Fe2S]- and six [4Fe4S]-clusters consistent with the experimental determination of 32 mol of Fe and 30 mol of acid labile sulfur. The enzyme catalyzed the exergonic reduction of NAD^+ with hydrogen as reductant only in the presence of flavin and ferredoxin. A $k_{M,\,app}$ for FMN of 6 μM and for ferredoxin of 12 μM was determined. The enzyme also catalyzed the endergonic reduction of ferredoxin with H_2 as reductant in a reaction that was also strictly dependent on NAD^+ and FMN. Spectral analyses revealed that ferredoxin and NAD^+ were reduced at the same time with a stoichiometry of 1:1. Apparently, the multimeric hydrogenase of *A. woodii* used the novel mechanism of electron bifurcation in which the endergonic reduction of ferredoxin with electrons derived from molecular hydrogen is coupled with the exergonic electron transfer from molecular hydrogen to NAD^+. The implications for the energy metabolism of acetogenic and other bacteria are discussed.

PSP061

Initial insights into the organohalide respiratory process of *Dehalococcoides* sp. strain CBDB1

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Microbial reductive dechlorination plays a crucial role in the detoxification of persistent halogenated compounds in contaminated environments. Several anaerobic bacteria like *Dehalococcoides* species are able to use a wide range of these contaminants as terminal electron acceptors in an anaerobic respiration with hydrogen as the so far sole known electron donor. This specialization is also reflected in the high number of putative reductive dehalogenase genes in the available genomes (e.g. 32 reductive dehalogenase homologous genes in *Dehalococcoides* sp. strain CBDB1 [1]).

To understand this exceptional lifestyle, we aim to elucidate the respiratory electron-transfer chain between the hydrogenase(s) and the reductive dehalogenase(s) including the identification and characterization of the electron-conducting components using *Dehalococcoides* sp. strain CBDB1 as a model organism. We have assigned an enzymatic function for one of the reductive dehalogenase homologous genes using native gel electrophoresis [2]. Also, we have now revised all cultivation, cell counting, cell harvesting as well as protein quantification and protein enrichment procedures to provide sufficient amounts of biomass for advanced biochemical experiments. Correlation of protein expression patterns with different halogenated electron acceptors using enzymatic activity tests and mass spectrometric analysis indicates the involvement of a series of reductive dehalogenase proteins in various dehalogenation reactions. On the other hand, several potential membrane soluble electron-conducting candidates were identified and are further studied by *in vitro* enzyme activity assays. We also started with a general analysis of putative respiratory chain components by liquid chromatography mass spectrometry (LC-MS/MS) on the protein level.

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PSP062

Characterisation of a peptidyl-prolyl-cis-trans-isomerase of *Corynebacterium glutamicum*

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Since the first report of the enzyme-driven cis-trans isomerization of petidyl-prolyl-bonds in polypetides, the peptidyl-prolyl-cis-trans isomerases (PPIases) were found to be present in almost all sequenced genomes to date. In eukaryotes PPiases play a major role in signal transduction of the immune system, thus the mechanisms and inhibitors are widely investigated. PPIases are classified in three distinct families reflecting their biochemical properties of binding to distinct classes of inhibitor molecules. In prokaryotes, however, many PPIases were investigated *in vitro*, but only in a few cases *in vivo* effects had been pursued. We here present evidence for the *in vivo* function of the prokaryotic PPiase FkpA of the soil bacterium and model microorganism *Corynebacterium glutamicum*. FkpA belongs to the family of FK-506 binding proteins (FKBPs) which are inhibited by FK-506 (Tacrolimus).
At temperatures below 25°C and above 35°C FkpA has a positive effect on citrate synthase (CS) activityin vitroand delayed aggregation of CS at 37°C and above. In vivo, deletion of fkpA leads to decreased cell growth and the specific CS activities of *C. glutamicumΔfkpA* were found to be reduced by about 40%, when cells were cultivated in synthetic media containing either glucose or acetate as carbon source. DNA microarray analyses comparing the transcriptomes of *C. glutamicumΔfkpA* and the wild type revealed, amongst others, that the RNA level of lactate dehydrogenase (*ldh*) was 10-fold increased in the deletion mutant, whereas the specific Ldh-activity was solely slightly increased, which could be interpreted as a direct effect of missing PPiase activity. Taken together we provide evidence that PPiases play a major role in protein stability and folding and thereby modulate enzyme activity in prokaryotes. Additionally, PPiases may broaden the optimal temperature range of their substrate enzymes.

PSP063

Differentiation of respiratory molybdopterin-containing oxidoreductases: insight multiple functions, structures and genetic composition

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Bacteria commonly perform anaerobic respiration driven by electron transport chains. Such respiratory chains consist of membrane-bound and soluble electron transport proteins that are involved in proton motive force generation. A high number of these proteins are molybdopterin-containing oxidoreductases that belong to the dimethyl sulfoxide (DMSO) reductase family. From an evolutionary point of view, it is assumed that these molybdoproteins are ancient enzymes involved, for example, in conversion of sulfur containing compounds.
Due to the homology within this group of molybdoenzymes it is challenging to predict the substrate range of such enzymes from genome data only. In particular, high similarities exist within the hydrophilic subunits of the DMSO reductase family comprising catalytic subunits containing the MGD ligated molybdenum ion as well as electron transfer subunits containing Fe/S clusters. In contrast the quinone- / quinol-reactive membrane subunits show differences [1,2].
The aim of this study was to functionally discriminate molybdoenzymes containing membrane subunits of the PsrC/NrfD family. The genome of the model organism *Wolinella succinogenes* encodes 11 different respiratory molybdoenzymes [3], several of which have unknown functions. Five of them are predicted to contain subunits of the PsrC/NrfD

family, but only one of these, the polysulfide reductase, has been characterized in the past [4].
Enzymatic, genetic and structural approaches were used to explore the functions of molybdoenzymes in anaerobic respiration of *Wolinella succinogenes*, focusing mainly on substrate specificities towards sulfide, polysulfide, thiosulfate, tetrathionate, dimethyl sulfoxide, trimethylamine N-oxide, chlorate, perchlorate, selenate and arsenate.
Results confirm the present of a sulfide dehydrogenase, a second polysulfide reductase and an arsenate reductase coded by different operons.
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PSP064
Springs of life in a "Dead Sea"

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The Dead Sea is a terminal lake located on the border between Jordan and Israel. Until 1979 the Dead Sea was a meromictic lake with hypersaline, anoxic and sulfidic deep waters and a seasonally varying mixolimnion (Anati *et al*., 1987). Since then the water column is mixed and the salinity is approximately 350 g L^{-1}. Surface springs running into the Dead Sea have been documented along the western and eastern shore of the lake (LaronneBen-Itzhak and Gvirzman, 2005, Lensky *et al*., 2005). In September 2009 we detected a large system of underwater springs on the western coast of the Dead Sea. Due to their deep emergence (down to 30 m depth), these springs cannot be detected by visual inspection of surface waters or by low resolution aerial thermal imaging.
In a preliminary research we explored the biological potential of these springs. Whereas the water column and sediments of the Dead Sea are largely devoid of life, dense microbial mats were found in the vicinity of the freshwater springs. The mats harbor a great diversity of microorganisms as shown by microscopy, hyperspectral imaging and next generation sequencing. Besides heterotrophic bacteria the mats consisted of diatoms, green sulfur bacteria, cyanobacteria, sulfide oxidizing and sulfate reducing bacteria. The microbial mats may thrive on high concentrations of organic matter and sulfide from the springs, and local primary production. In-situmicrosensor measurements using oxygen and sulfide electrodes as well as measurements of sulfate reduction give first insights into the biological activities in these unique microbial consortia. We found evidence for an active sulfur cycle within the microbial mats, no activities were found in reference Dead Sea habitats. CO_2 fixation was detected by stable isotope incubations. Fluctuations in temperature indicate a changing flow of the freshwater streams thus the microorganisms have to cope with fast changes of salinity from hypersaline to freshwater.
We will present new details on the microbial community composition and activity of these oases of life in an otherwise extremely hostile environment.
Anati DA, Stiller M, Shasha S, Gat JR (1987) Changes in the thermo-haline structure of the Dead Sea: 1979-1984. Earth Planet Sci Lett,84: 109-121.
Laronne Ben-Itzhak L, Gvirtzman H (2005) Groundwater flow along and across structural folding: an example from the Judean Desert, Israel. J Hydrol,312: 51-69.
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QDV1-FG
Development of hygiene monitoring media with non-animal origin – bachelor thesis written in industry

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Within the pharmaceutical hygiene monitoring microbiological testing of the air, personnel and surfaces are carried out traditionally with Tryptic Soy Agar (TSA) and/or Sabouraud Dextrose Agar (SDA). Both media are containing peptones from animal origin – casein peptone in TSA and meat and casein peptone in SDA.
Due to the BSE risk of peptone from animal origin, the use of raw materials from non-animal origin would be advantageous for microbiological hygiene monitoring in pharmaceutical plants.
The aim of this bachelor thesis included the development of a new TSA and a new SDA both based on strictly non-animal originated raw materials. Testing of the microbiological growth performance followed the specifications given in the pharmacopoeias (EP/USP/JP). Also common environmental isolates were included during testing different formulations. TSA and SDA from EP/USP/JP formulation were used as references during all steps of the development. The new TSA and SDA from non-animal origin had to show the same or better performance characteristics as the traditional formulations using animal-origin peptones.
In addition to the presentation of the technical part, a short summery of experiences writing the bachelor thesis in industry will be given.
1.European Pharmacopoeia 7.0 (2011) chapter 2.6.12 and 2.6.13.
2.United States Pharmacopeia XXXIV (2010) chapter <61> and <62>.
3.Japanese Pharmacopeia 16th Edition (2011) chapter 4.05.

QDV2-FG
Erfahrungen als EMbaRC-Stipendiatin bei der BCCM/LMG in Gent – Nutzen für das eigene Forschungsprojekt"

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Das „Training und Outreach Programme" des „European Consortium of Microbial Resource Centres" (EmbaRC) ermöglichte mir die Teilnahme am Kurs: „Taxonomy, identification and typing of prokaryotes" im Labor für Mikrobiologie der „Belgian Co-ordinated Collections of Micro-organisms" (BCCM/LMG) in Gent, Belgien.
Dabei handelte es sich um ein zweiwöchiges Training, durch das ich einen erweiterten Einblick in die polyphasische Taxonomie und die Anwendung spezieller Techniken zur Klassifizierung und Identifizierung von Bakterien erhielt. Diese Themen wurden im Vorfeld auf mein Projekt zugeschnitten und somit im Hinblick auf Bacillus-Stämme behandelt, weil ich mich in meinem Projekt an der Beuth Hochschule für Technik in Berlin in der AG Prowe mit der Entwicklung eines Nachweises des Bacillus-Genus und gleichzeitiger Identifikation einzelner Bacillus-Spezies mittels real-time PCR beschäftige. Dazu wurde der Zugriff auf Stämme der BCCM/LMG Sammlung gewährt.
Während des Programms erhielt ich eine praktische und theoretische Vertiefung meines Wissens zu Detektionsmethoden von Bakterien. Diese Inhalte wurden im Labor durch die Kultivierung von Bacillus-Stämmen und die Identifizierung derselben mittels biochemischer Tests vermittelt. Zudem wurden verschiedene DNA Extraktionsmethoden und eine Qualitätskontrolle der Extrakte durchgeführt. Die theoretischen Inhalte wurden durch Vorlesungen zur Taxonomie und Klassifizierung von Bakterien, zu 16S rDNA Sequenz-Datenbanken und, aufgrund der projektbezogenen Inhaltsanpassung, zur real-time PCR vertieft.
Durch dieses Programm wurde der erste Kontakt zwischen Wissenschaftlern beider Institutionen hergestellt und zukünftige Kooperationen werden angestrebt.
Mein Dank gilt BCCM für die exzellente Organisation sowie EmbaRC für die Finanzierung durch den Transnational Access Grant.

QDV3-FG
Das Berufsfeld des Mikrobiologen in einer Behörde – Diagnostik im Rahmen der biologischen Arbeitssicherheit

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Unsere Aufgabe im Arbeitsschutz im Bereich der mikrobiologischen Diagnostik besteht vorran-gig darin, sogenannte „Biologische Arbeitsstoffe", worunter nach der Biostoff-Verordnung Mik-roorganismen einschließlich gentechnisch veränderter Mikroorganismen, Zellkulturen und hu-manpathogene Endoparasiten sowie auch mit transmissiblen spongiformen Enzephalopathien assoziierte Agenzein verstanden werden, in der Luft am Arbeitsplatz oder in unterschiedlichsten Materialproben nachzuweisen, ggf. ihre Konzentration zu bestimmen und die mögliche Auswir-kung ihres Vorhandenseins im Rahmen der Ausübung einer beruflichen Tätigkeit zu beurteilen.
Biostoff-Verordnung ? siehe
http://www.bmas.de/DE/Service/Gesetze/biostoffv.html
Die Ergebnisse aus solchen Untersuchungen dienen dazu Arbeitsplätze mit Blick auf eine mög-liche Gefährdung der Beschäftigten bei Ausübung ihrer Tätigkeit durch eine Exposition gegen-über biologischen Arbeitsstoffen zu beurteilen. Das ist eine allgemeine Forderung der Biostoff-Verordnung, der jeder Arbeitgeber nachkommen muß, dessen Beschäftigte Tätigkeiten aus-üben, bei denen biologische Arbeitsstoffe freigesetzt werden wodurch sie direkt mit diesen in Kontakt kommen können.

Bei den verschiedenen Unfallversicherungsträgern (z. B. Unfallkassen des Bundes und der Länder oder gewerbliche Berufsgenossenschaften wie die BG RCI = Berufsgenossenschaft der chemischen Industrie) fließen unsere Ergebnisse in branchenbezogene Handlungsanleitungen, Informationsschriften und Regeln ein. Mikrobiologische Untersuchungen werden von diesen Institutionen auch durchgeführt, um den Zusammenhang zwischen dem Auftreten einer berufs-bedingten Infektionskrankheit oder auch allergisch oder toxisch bedingten Atemwegserkrankung und dem Vorhandensein von bestimmten Infektionserregern, Allergenen oder toxisch wirkenden Substanzen mikrobiologischen Ursprungs aufzuzeigen.
In verschiedenen staatlichen Gremien, wie dem Ausschuss für biologische Arbeitsstoffe (ABAS) mit seinen verschiedenen Unterausschüssen und Arbeitskreisen (http://www.baua.de/de/Themen-von-A-Z/Biologische-Arbeitsstoffe/ABAS/ABAS.html), werden Ergebnisse aus mikrobiologischen Untersuchungen herangezogen um technische Re-geln zu biologischen Arbeitsstoffen zu erstellen
(http://www.baua.de/de/Themen-von-A-Z/Biologische-Arbeitsstoffe/TRBA/TRBA.html).
Die Untersuchungen zur Gefährdungsbeurteilung von Beschäftigten durch Exposition gegenüber biologischen Arbeitsstoffen am Arbeitsplatz erfolgen mit Hilfe eines klassischen mikrobio-logischen Verfahrens: der Anzucht von Mikroorganismen (i. d. R. Bakterien, Hefen und Schimmelpilze) auf Nährböden und Bestimmung einer Koloniezahl als Anzahl Kolonie bildender Ein-heiten, KBE bzw. englisch: colony forming units, cfu) bezogen auf ein Aliquot des Materials, aus dem die Organismen isoliert wurden (häufig Luft, aber auch Wasser u. a. Flüssigkeiten oder unterschiedliche feste Materialien, die als Mikroorganismenquelle in Betracht kommen).
Insbesondere wenn es um einen raschen Nachweis des Vorhandenseins von Mikroorganismen überhaupt oder aber um den gezielten Nachweis ausgewählter Organismen wie z. B. von Legionella pneumophila, Serogruppe 1 aus dem Befeuchterwasser einer sogenannten Klimaan-lage oder von Mycobacterium immunogenum im Kühlschmierstoff eines Metallfertigungsbetrie-bes geht, kommen auch moderne molekularbiologische Verfahren oder Fluoreszenzmikroskopi-sche Techniken zum Einsatz.

QDV4-FG

From academia to industry, and back: Microbiological research to make life easier, better and more beautiful

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The consumer goods producing industry represents an attractive employer for microbiologist, that are interested to use their knowledge and technological skills not only in the field of quality control, but also for research and development of novel products for very dynamic and profit-oriented markets.
The aim of this presentation is to provide some personal insight into the nature of the job of a microbiologist (with a strong background in molecular microbial ecology), working in the area of consumer goods, using the Henkel AG & Co. KGaA as an example. Henkel, headquartered in Düsseldorf / Germany, has about 48,000 employees worldwide and counts among the most internationally aligned German-based companies in the global marketplace. The company has three globally operating business sectors: Laundry & Homecare, Cosmetics & Toiletries and Adhesive Technologies.
Using selected studies from the fields of skin microbiology [1], household hygiene [2], and rapid methods for quality control, the talk will provide a brief overview of the diversity of factors and topics driving microbiological projects at a company like Henkel. In addition, some personal comments on the requirements for a successful application and some general pros and cons of starting a career in the consumer goods producing industry will be given. Finally, the job profile of a professor working at an University of Applied Sciences will be presented as a career option that combines several aspects of an industrial and academic career.
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RSV001

Functional interaction of the *Escherichia coli* transporters DctA and DcuB with the sensor kinase DcuS

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Escherichia coli can use C_4-dicarboxylates as carbon and energy sources for aerobic or anaerobic respiration. The two component system DcuSR activates the transcription of *dctA* (succinate import), *dcuB* (fumarate-succinate antiport), *fumB* (fumarase) and *frdABCD* (fumarate reductase) in the presence of C_4-dicarboxylates [1]. DcuSR consists of the membrane integral sensor kinase DcuS and the cytoplasmic response regulator DcuR. DcuS contains a periplasmic PAS domain which responds to the presence of C_4-dicarboxylates.
DctA is the main transporter for the uptake of C_4-dicarboxylates under aerobic conditions. Under anaerobic conditions the DcuB transporter catalyses a fumarate/succinate antiport which is essential for the fumarate respiration [1]. DctA and DcuB function as essential co-sensors of DcuS. Deletion of the carriers causes constitutive activation of DcuSR [2, 3].
Overproduction of DctA under anaerobic conditions allowed it to replace DcuB in co-sensing, suggesting that DcuB and DctA are functionally equivalent in this capacity. Interaction of the integral membrane protein DcuS with DctA and DcuB was analysedin vivowith a bacterial two-hybrid system based on theBordetella pertussisadenylate cyclase (BACTH) and by fluorescence resonance energy transfer (FRET). Direct interaction of DctA and DcuB with DcuS was observed. DctA contains a cytosolic amphipathic helix following its last transmembrane helix. Mutational analysis demonstrated the importance of this helix in interactions, co-sensing and transport.
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RSV002

YhbJ - a novel RNA binding protein functions as mediator of signal transductionin the hierarchically acting GlmYZ sRNA cascade

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In *Escherichia coli*, expression of key enzyme glucosamine-6-phosphate synthase (GlmS) is feedback-regulated by two homologous sRNAs, GlmY and GlmZ, in a hierarchical manner [1,2,3,4]. GlmS catalyzes formation of glucosamine-6-phosphate (GlcN6P), an essential precursor for cell wall biosynthesis. Depletion of cellular GlcN6P induces accumulation of GlmY by a post-transcriptional mechanism [5]. GlmY counteracts processing of GlmZ. Exclusively unprocessed GlmZ can base-pair with the *glmS* 5' UTR and activate translation. Subsequently, GlmS is synthesized and refills the GlcN6P pool in the cell. The mechanism of signal transduction from GlmY to GlmZ involves the novel RNA binding protein YhbJ. In mutants lacking *yhbJ* processing of GlmZ is abolished and *glmS* is chronically activated [1,4]. Here we show that GlmZ is processed by RNase E and that YhbJ and RNase E interact. Furthermore, YhbJ drastically stimulates the RNase E-dependent processing of GlmZ *in vitro* suggesting that YhbJ recruits GlmZ to its processing machinery. Indeed, YhbJ specifically binds both sRNAs *in vivo* and *in vitro* and this binding appears to be modulated by cellular GlcN6P levels. Upon GlcN6P-starvation GlmY accumulates and sequesters YhbJ thereby outcompeting GlmZ. Under these conditions GlmZ is protected from processing and able to activate *glmS* expression. Our data indicate that YhbJ could be a novel specificity factor guiding RNase E to its substrate GlmZ.
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RSV003

Structural insights into the redox-switch mechanism of HypR, a disulfide stress-sensing MarR/DUF24-family regulator of *Bacillus subtilis*

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Bacillus subtilis encodes redox-sensing MarR-type regulators belonging to the 1-Cys OhrR and 2-Cys DUF24-families that are conserved among bacteria and control virulence functions in pathogens via thiol-based redox-switches. While OhrR proteins respond to organic hydroperoxides, the DUF24-family senses electrophiles such as diamide, quinones or aldehydes [1]. Here, we characterize the novel redox-sensing MarR/DUF24-family regulator HypR (YybR) that is activated by disulfide stress caused by diamide and NaOCl in *B. subtilis*. HypR controls positively a flavin oxidoreductase HypO that confers protection against NaOCl stress [2]. The conserved N-terminal Cys14 residue of HypR has a lower pK_a of 6.4 and is essential for activation of *hypO* transcription by disulfide stress. HypR resembles a 2-Cys-type regulator that is activated by Cys14-Cys49' intersubunit disulfide formation. The crystal structures of reduced and oxidized HypR proteins were resolved revealing the mechanism of HypR activation. In reduced HypR a hydrogen-bonding

network stabilizes the reactive Cys14 thiolate that is 8-9 Å apart from Cys49'. HypR oxidation breaks these H-bonds, reorients the monomers and moves the major groove recognition alpha4 and alpha4' helices ~4 Å towards each other. This is the first crystal structure of a redox-sensing MarR/DUF24 family protein in bacteria that is activated by NaOCl stress. Since hypochloric acid is released by activated macrophages as major defense mechanism, related HypR-like regulators could function to protect pathogens against the host immune defense.

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RSV004

How the P_{II} protein from *Synechococcus* integrates metabolic with energy signals to control its targets.

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P_{II} signal transduction proteins have key functions in coordination of central metabolism by integrating signals from carbon, nitrogen and energy status of the cell. They bind the metabolites ATP, ADP and 2-oxoglutarate (2-OG) and control enzymes, transporters and transcription factors involved in nitrogen metabolism. Depending on its effector molecule binding status, P_{II} from *Synechococcus* elongatus binds a small protein termed PipX, which is a co-activator of the transcription factor NtcA, and regulates the key enzyme of the cyclic ornithine pathway, N-acetyl-L-glutamate kinase (NAGK). P_{II} binds ATP and 2-OG in a synergistic manner, with the ATP-binding sites also accepting ADP. Different ADP/ATP ratios strongly affect the properties of P_{II} signaling including 2-OG binding and interactions with its target proteins. ADP modulates P_{II} signaling to the receptor NAGK primarily at low 2-OG levels and antagonises the inhibitory effect of 2-OG for P_{II}-PipX interaction. Apparently P_{II} has a fine-tuned mechanism of sensing both changing energy charge and carbon/nitrogen balance at the same time.

Fokina O., Chellamuthu VR., Zeth K., & Forchhammer K. (2010) A novel signal transduction protein P_{II} variant from *Synechococcus elongatus* PCC 7942 indicates a two-step process for NAGK-P_{II} complex formation. *J. Mol. Biol.* 399:410-421.
Fokina O., Herrmann C. & Forchhammer K. (2011) Signal transduction protein P_{II} from *Synechococcus elongatus* PCC 7942 senses low adenylate energy charge *in vitro*. *Biochem. J.* 440:147-56.

RSV005

Post-translational modification determines the substrate specificity of a carboxylic acid-coenzyme A ligase

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In anaerobic bacteria most aromatic growth substrates are converted to the central intermediate benzoyl-CoA. E.g., in case of benzoate and *p*-hydroxybenzoate degradation, the initial steps are usually catalyzed by individual, highly specific carboxylic acid CoA ligases[1]. We established a first genetic system for the obligately anaerobic model organism *Geobacter metallireducens* and disrupted the gene encoding benzoate-CoA ligase (*bamY*) as a proof of principle. This enzyme is highly specific for benzoate as substrate[2]. Unexpectedly, a *bamY* mutant was still able to grow on benzoate with a growth rate similar to that of the wild type. In agreement, we identified a previously unknown succinyl-CoA:benzoate CoA transferase, which obviously fully compensated for the *bamY* knockout. Surprisingly, the *bamY* mutant was no longer able to utilize *p*-hydroxybenzoate as carbon source, although isolated BamY was unable to activate *p*-hydroxybenzoate. Growth on *p*-hydroxybenzoate was observed again in the presence of a plasmid expressing intact *bamY*, suggesting that *bamY* is involved in *p*-hydroxybenzoate catabolism. Strictly dependent on acetyl-CoA, incubation of purified BamY with dialyzed extract of cells grown on *p*-hydroxybenzoate converted BamY to a *p*-hydroxybenzoate-CoA ligase. Results of MS-analysis of tryptic digests suggest that different patterns of N^{ε}-lysine acetylation were responsible for altered substrate specificities of BamY. Though N^{ε}-acetylation of active site lysines has been reported to switch the activity of carboxylic acid CoA ligases off[3], the modulation of substrate specificity via post-translational N^{ε}-lysine acetylation was previously known.

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RSV006

Hot signal transduction in the thermoacidophilic creanarchaeum *Sulfolobus acidocaldarius*

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Posttranslational modifications are of major interest for the regulation of cellular processes. Reversible protein phosphorylation is the main mechanism to control the functional properties of proteins in response to environmental stimuli [1]. In the 80's protein phosphorylation has been demonstrated in the third domain of life, the Archaea [2]. However, so far only few phospho(p) proteins were identified and few protein kinases and protein phosphatases were investigated. The archaeal phosphorylation machinery in general resembles more the eucaryal (Ser, Thr and Tyr phosphorylation) than the bacterial machinery (two- and one-component systems, Asp and His phosphorylation). Bioinformatic analysis revealed that Ser, Thr and Tyr phosphorylation is ubiquitous in Archaea, whereas two- and one-component systems are only present in the euryarchaeota (e.g. CheA/CheY in *Halobacterium salinarium*) [1,3].
Model organism of this study is the thermoacidophilic Creanarchaeon *Sulfolobus acidocaldarius*, with optimal growth at 78°C and pH of 2-3 [4]. Bioinformatic investigation revealed that *S.acidocaldarius* harbors twelve protein kinases and two protein phosphatases [1]. Nine of the twelve identified protein kinases (PK) show high sequence similarity to eukaryal type like protein kinases and the remaining three to atypical protein kinases. The two protein phosphatases (PP) show similarity to protein tyrosine phosphates (PTP) and protein phosphatases (PPP). Furthermore, *Sulfolobus* species itself have an unusual high PK to PP ratio (12:2) compared to other archaea (3:1 to 1:1) [1]. First analysis of the p-proteome revealed a high no. of p-proteins and a high no. of p-Tyr (Ser 31.8%, Thr 24.8%, Tyr 43.3%). The detected p-proteins are found in all major arCOG categories.
In order to investigate signal transduction in *S. acidocaldarius* we cloned and characterized the PP2A catalytic subunit from *S. acidocaldarius*. Until know, all investigated archaeal PPPs are members of the PP1-arch [5-7] and so far no member of the PP2-arch was characterized. This is the first detailed characterization of an archaeal PP2A. The current understanding of signal transduction in *S. acidocaldarius* with focus on the PP2A will be presented.

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RSV007

A novel LuxR-based cell-to-cell communication system in the entomopathogen *Photorhabdus luminescens*

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Cell-to-cell communication via acyl-homoserine lactones (AHL) is well studied in many Gram-negative bacteria. The prototypical communication system consists of a LuxI-type autoinducer synthase and a LuxR-type receptor that detects the endogenously produced signal. The symbiotic and entomopathogenic enteric bacterium *Photorhabdus luminescens* harbors 39 LuxR-like receptors, but lacks any LuxI-type autoinducer synthase and is unable to produce AHL. It is unclear whether *P. luminescens* uses these orphan LuxR homologues for the detection of exogenous or endogenous signals. In this study we demonstrate that *P. luminescens* does engage in endogenous LuxR-based cell-cell communication. We show that one of the LuxR homologues, Plu4562 (PluR), detects an endogenously produced signaling molecule (PLAI-1) that is not an AHL but, rather, a 2-pyrone derivative named photopyrone. We also show that PluR positively regulates the expression of the *plu4568-plu4563* operon, encoding genes involved in cell clumping. However *plu4568-plu4563* is not responsible for the production of PLAI-1 and the nature of the clumping factor produced by this operon remains unidentified. We also show that the LysR-type regulator HexA, which is a global repressor of symbiosis genes in *P. luminescens*, represses *plu4568-plu4563* expression. This suggests that the *plu4568-plu4563* operon may be involved in the mutualistic interaction between *P. luminescens* and the nematode. Indeed we have shown that colonization of the symbiotic partner *Heterorhabditis bacteriophora* by a *P. luminescens* Δ*pluR* mutant does appear to be

reduced. Summarizing, *P. luminescens* does produce a novel cell-cell signaling molecule, PLAI-1, that controls the expression of the *plu4568-plu4563* operon in a manner that is dependent on the orphan LuxR-like regulator, PluR.

RSV008

α-Hydroxyketone-mediated signal transduction in *Legionella pneumophila*

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The causative agent of Legionnaires' disease, *Legionella pneumophila*, is a parasite of environmental protozoa. *L. pneumophila* employs a biphasic life cycle to replicate within and spread to new host cells. The switch from the replicative to the transmissive (virulent) state is coordinated by a complex regulatory network, including signaling through endogenously synthesized small molecules ("autoinducers") in a process termed "quorum sensing". *L. pneumophila* produces and likely responds to α-hydroxyketone signaling molecules [1].
The *lqs* (*Legionella* quorum sensing) gene cluster harbors homologs of the *Vibrio cholerae cqsAS* genes, i.e. *lqsA* and *lqsS*, flanking a gene called *lqsR*. The autoinducer synthase LqsA catalyzes the production of LAI-1 (*Legionella* autoinducer-1, 3-hydroxypentadecan-4-one), which is presumably recognized by the sensor kinase LqsS, and LqsR is a novel putative response regulator that controls bacterial virulence and replication. Functional studies and transcriptome analysis revealed that *lqsR*, *lqsS* and *lqsA* regulate *L. pneumophila*-host cell interactions, extracellular filaments and a genomic "fitness island" [1].
Through bioinformatic analysis an "orphan" homolog of the sensor kinase *lqsS* was identified in the *L. pneumophila* genome and termed *lqsT*. *L. pneumophila* lacking *lqsT* is impaired for virulence and intracellular replication. Biochemical studies indicate that LqsS and LqsT are sensor kinases, which interact with the putative response regulator LqsR. These results suggest that *L. pneumophila* responds to α-hydroxyketone signals sensed by LqsS and LqsT, which converge on LqsR. Using biochemical, genetic and cellular microbial approaches, the role of LqsS, LqsT and LqsR in α-hydroxyketone-mediated signal transduction and gene regulation is further analyzed in detail.
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RSV009

A high-frequency mutation in *Bacillus subtilis*: Requirements for the decryptification of the *gudB* glutamate dehydrogenase gene

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Common laboratory strains of *Bacillus subtilis* encode two catabolic glutamate dehydrogenases, the enzymatically active protein RocG and the cryptic enzyme GudB that is inactive due to a duplication of three amino acids in its active centre (1, 2). The inactivation of the *rocG* gene results in poor growth of the bacteria on complex media due to the accumulation of toxic intermediates. Therefore, *rocG* mutants readily acquire suppressor mutations that decryptify the *gudB* gene. We showed that the decryptification occurs by a precise deletion of one part of the nine base pair direct repeat that causes the amino acid duplication. The deletion occurs at the extremely high rate of 10^{-4} (3). This is the highest mutation rate that was observed for a specific allele in *B. subtilis*. Mutations affecting the integrity of the direct repeat result in a strong reduction of the mutation rate; however, the actual sequence of the repeat is not essential (3). We also demonstrated that the mutation rate of *gudB* is not affected by the position of the gene on the chromosome. When the direct repeat was placed in the completely different context of an artificial promoter, the precise deletion of one part of the repeat was also observed, but the mutation rate was reduced by three orders of magnitude. Thus, transcription of the *gudB* gene seems to be essential for the high rate of the appearance of the *gudB1* mutation. This idea is supported by the finding that the transcription-repair coupling factor Mfd is required for the decryptification of *gudB*. The Mfd-mediated coupling of transcription to mutagenesis can be regarded as a built-in precaution that facilitates the accumulation of mutations preferentially in transcribed genes (4).
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RSV010

The PpsR Protein in *Rhodospirillum rubrum*: A major metabolism coordinator

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Being a facultative anoxygenic photosynthetic bacterium, *Rhodospirillum rubrum* is able to adapt to various environmental conditions. Especially, the availability of oxygen demands for a precise regulatory response in purple bacteria. Under anaerobic conditions anoxygenic photosynthesis is a very important energy source, but under aerobic conditions the photosynthetic membranes (PM) can produce highly toxic reactive oxygen species. The switch between aerobic respiratory metabolism and anaerobic photosynthetic metabolism is a great regulatory challenge for all purple bacteria so that many regulators are involved and several interesting strategies have evolved.
In most purple bacteria, two major regulatory systems, the RegB/RegA system and the PpsR system control photosynthetic gene expression. Both systems are well investigated in Rhodobacter species, often compared with *R. rubrum*. Basically, the RegB/RegA system activates photosynthetic gene expression when oxygen concentration is low, whereas PpsR represses the expression under aerobic conditions.
Generally, in purple bacteria, the application of semiaerobic dark conditions results in basal expression of photosynthetic genes. However, so far only observed in *R. rubrum*, this PM-level can be strongly increased by the addition of fructose or reduced glutathione to the culture medium (1). It is tempting to assume that both compounds provide additional reducing equivalents thereby affecting identical redox regulatory pathways. Furthermore, no homologues of RegB/RegA can be found by gene sequence analysis in *R. rubrum*. This suggests a more central role of the PpsR-homologue in this bacterium.
In this work, we show that PpsR in *R. rubrum* is most likely an activator protein for the photosynthetic genes. We used PpsR from heterologous expression in *E. coli* for DNA-binding assays and overexpression strains for the elucidation of the role of PpsR in *R. rubrum*. The significance of PpsR is underlined by the fact that no stable deletion strain could be created.
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RSV011

Fine-tuning of sulfur metabolism by a peptide-coding sRNA in the photooxidative stress response of *Rhodobacter*

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The photosynthetic model organism *Rhodobacter sphaeroides* faces photooxidative stress due to the bacteriochlorophyll-mediated generation of singlet oxygen ($^1O_2$) in the light. In recent years several regulatory factors were identified which guide adaptation to these harmful conditions. The alternative sigma factor RpoE, which is on top of $^1O_2$-dependent regulation, induces amongst others the 219 nt long sRNA RSs0019 (1). RSs0019 contains a small ORF (150 nt), which was shown to be strongly translated under $^1O_2$ stress. Overexpression of RSs0019 combined with microarray analysis suggested a role in fine-tuning of sulfur metabolism. To distinguish between peptide- versus sRNA-driven effects, an RSs0019 variant with an internal stop-codon was designed and compared to the genuine sRNA by real time RT-PCR. These experiments both verified the microarray data and suggested RSs0019 to be a bifunctional RNA. To gain further insights into the regulatory mechanisms, more precisely the RNA binding and potential translational effects of RSs0019, we made use of a *lacZ*-based *in vivo* reporter system for *Rhodobacter*. In this context, mutational analyses of both RSs0019 and potential target mRNAs were constructed to uncover distinct interactions and outputs. Our data provide evidence that RSs0019 is a riboregulator which encodes a small peptide and fine-tunes the sulfur metabolism in *Rhodobacter* when sulfur stress originates after $^1O_2$ generation.
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RSV012

The conserved sRNA scr5239 controls DagA expression by translational repression

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We were interested in the identification and characterization of sRNAs in *Streptomyces coelicolor*. To find such transcripts we performed deep sequencing of the *S. coelicolor* transcriptome. That way we identified 63 new non-coding RNAs and confirmed the expression for 11 [1].
One sRNA - called scr5239 - found in the sequencing data especially attracted our interest because of its high degree of sequence and structure conservation. It is a 159 nt long sRNA with >90% conservation in 15 streptomyces genomes and is constitutively expressed under most

conditions tested. Its 2D structure - as validated by enzymatic probing - consists of five independent stem-loops.
Overexpression or knockdown of scr5239 results in distinct macroscopic phenotypes. The scr5239 overproduction strain can not express the agarase DagA and therefore cannot use agar as carbon source (Fig. 1A). Interestingly, the level ofdagAmRNA is not influenced by scr5239. Nevertheless, we identified a direct and specific interaction of the dagA mRNA with scr5239 using competitive gel mobility shift assays and chemical probing. The interaction occurs in the coding region of the agarase mRNA ~ 40 nt downstream of the start codon possibly blocking translation [2].
DagA, however, is not the only target of scr5239. Currently, we investigate possible further targets of scr5239 regulation in the central metabolism of *S. coelicolor*.

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RSV013

Two hybrid histidine kinases utilize inter- and intra-protein phosphorylation to regulate developmental progression in *Myxococcus xanthus*

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Signal transduction in bacteria is primarily mediated by histidine-aspartate (His-Asp) phosphorelay [often termed two-component signal transduction (TCST)] proteins. In the paradigm two-component system, the signaling process is mediated by two proteins: a histidine protein kinase (HPK) and a response regulator (RR). Signal perception by the HPK leads to phosphorylation of an invariant histidine residue, which then serves as a phosphoryl donor for the aspartic acid residue in the RR. RR phosphorylation modulates a cellular response. In addition to these simple two-component systems, the highly adaptable histidine kinase and receiver modules can be arranged into more sophisticated signaling systems which provide additional sites for regulation, and integration of disperse signals into a common response. These more complex systems are favored in microorganisms with complex lifestyles.
Upon nutrient limitation, *Myxococcus xanthus* undergoes a developmental process in which the cells of the swarming community aggregate into multicellular fruiting bodies and then differentiate into environmentally resistant spores. *M. xanthus* encodes a large repertoire of His-Asp phosphorelay proteins, many of which are involved in complex signaling pathways. Several of these complex signaling systems have been shown to be negative regulators of developmental progression, because the respective mutants develop earlier than the wild type.
In this study, we demonstrate that two orphan HyHPK, EspA and EspC, intimately function together in a single signaling system. Using a combination of genetic, biochemical, and bioinformatic analyses, we demonstrate that EspC's kinase region does not act as a phosphor donor for the Esp system. Interestingly however, EspA's kinase performs intra- and inter-molecular phosphorylation of both its own and EspC's receiver domain, which together control developmental progression. Additionally, we demonstrate that the Esp system regulates developmental progression by controlling the proteolytic turnover of MrpC an important developmental transcription factor. We speculate that this regulated degradation of MrpC ensures a gradual accumulation of MrpC which is necessary for coordinated fruiting body formation.

RSV014

Studies of an Fnr-like transcriptional regulator in *Gluconobacter oxydans* 621H

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The strictly aerobic α-proteobacterium *Gluconobacter oxydans* is used for a wide variety of industrial applications such as vitamin C synthesis. One special characteristic is the incomplete oxidation of substrates like sugars or sugar alcohols in the periplasm. Despite its industrial importance, knowledge of the metabolism of *G. oxydans* and its regulation, especially concerning the sugar metabolism, is still very scarce. Transcriptional regulators participating in energy and redox metabolism have not been described yet. *In silico* analysis of the genome sequence revealed 117 genes from 38 different regulator families coding for putative transcriptional regulators (TRs).
Concerning the genomic proximity to important genes of the carbon metabolism or the potential function of the members of the TR repertoire, we selected candidates for further examination. As a consequence of TR deletion mutant studies we have chosen an Fnr-like regulator, GOX0974, for further characterisation. Fnr (fumarate-nitrate reduction regulator) in *Escherichia coli* is a switch between aerobic and anaerobic respiration. However, *G. oxydans* is strictly aerobic and so far no biochemistry for anaerobic respiration has been identified. With regard to these facts, a different function of GOX0974 is very likely that does not involve the switch to anoxic metabolism. The characterisation of this regulator included microarray and physiological analyses of ΔGOX0974 to identify target genes and phenotype. Additionally the biochemistry of this protein was studied, such as regulator activity and spectral properties. As a result, these experiments gave evidences for a new function of an Fnr-like protein.
The experimental studies of this regulator are the first presented of a transcriptional regulator from *Gluconobacter oxydans*.

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RSV015

$EIIA^{Ntr}$ of the nitrogen phosphotransferase system regulates expression of the *pho* regulon via interaction with histidine kinase PhoR in *Escherichia coli*

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In addition to the phosphotransferase system (PTS) dedicated to sugar transport, many Proteobacteria possess the paralogous PTS^{Ntr}. In the PTS^{Ntr} phosphoryl-groups are transferred from PEP to protein $EIIA^{Ntr}$ via the phosphotransferases EI^{Ntr} and NPr. The PTS^{Ntr} has been implicated in regulation of diverse physiological processes in different species (1). In *E. coli* PTS^{Ntr} plays a role in potassium homeostasis. In particular, $EIIA^{Ntr}$ increases expression of the genes encoding the high-affinity K^+ transporter KdpFABC. To this end, $EIIA^{Ntr}$ binds to and stimulates activity of histidine kinase KdpD, which in turn controls expression of *kdpFABC* (2). Here we show that the genes belonging to the phosphate (*pho*) regulon are likewise regulated by PTS^{Ntr}. The *pho* regulon is activated by the two-component system PhoR/PhoB under conditions of phosphate starvation (3). However, maximum expression of the *pho* genes requires $EIIA^{Ntr}$. The data reveal a direct interaction between $EIIA^{Ntr}$ and PhoR that ultimately stimulates phosphorylation of response regulator PhoB. Thus, the PTS^{Ntr} modulates the activity of two central sensor histidine kinases by direct interaction.

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RSV016

A *Pseudomonas putida* bioreporter strain for the detection of alkylquinolone-converting enzymes

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Pseudomonas aeruginosa is an opportunistic pathogen which regulates its virulence via a complex quorum sensing (QS) network. This network incorporates *N*-acylhomoserine lactones as well as 2-heptyl-3-hydroxy-4(1*H*)-quinolone (the *Pseudomonas* quinolone signal, PQS) and 2-heptyl-4(1*H*)-quinolone (HHQ). PQS and HHQ belong to the over 50 different 2-alkyl-4(1*H*)-quinolone (AQ) compounds which are produced by *P. aeruginosa*. They differ mainly in the length and degree of saturation of the alkyl chain and the presence or absence of a hydroxyl substituent at the C3-position [1]. Both PQS and HHQ act as the effectors of the LysR-type transcriptional regulator PqsR and operate as autoinducers in QS [2, 3].
We constructed the bioreporter strain *P. putida* KT2440 [pBBR1-*pqsR*-P_{pqsA}::*lacZ*] which constitutively expresses the *pqsR* gene, whereas the *lacZ* reporter gene is fused to the PqsR-responsive *pqsA* promoter. Therefore, β-galactosidase activity is a function of the PqsR-stimulated transcription, which is dependent on the concentration of HHQ or PQS. The bioreporter strain is highly sensitive for HHQ (EC_{50} 1.44 ± 0.23 μM) and PQS (EC_{50} 0.14 ± 0.02 μM).
To test whether the bioreporter strain can be used for the detection of AQ-degrading enzymes, the *hod* gene coding for 1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase was expressed in the bioreporter. Hod is an enzyme involved in the quinaldine (2-methylquinoline) degradation pathway of *Arthrobacter nitroguajacolicus* Rü61a and catalyzes the cleavage of 1*H*-3-hydroxy-4-oxoquinaldine to carbon monoxide and *N*-acetylanthranilate. It has been shown that Hod is also active towards the structurally related PQS, forming carbon monoxide and *N*-octanoylanthranilate [4].
P. putida KT2440 [pBBR1-*pqsR*-P_{pqsA}::*lacZ*] harboring pME6032-*hod* was cultivated in the presence of different PQS concentrations. The co-expression of *hod* significantly decreased the β-galactosidase activity in comparison to the corresponding control strain which contained the pME6032 vector. These results provide proof of principle that the bioreporter strain will be useful for the screening of AQ-converting enzymes.

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RSP001

Development of *in vitro* transcription system for *Corynebacterium glutamicum*

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In vitro transcription analysis is a powerful tool to study various aspects of transcriptional regulation of gene expression. We have developed the first *in vitro* transcription system for *Corynebacterium glutamicum*, a producer of amino acids used in biotechnological processes. A bacterial RNA-polymerase (RNAP) holoenzyme consists of a five-subunit (α_2, β, β´, ω) core and a dissociable sigma subunit (factor), which is responsible for the recognition of specific promoter DNA sequences. The genome of *C. glutamicum* codes for 7 sigma subunits of RNAP. Most of the *C. glutamicum* promoters driving transcription of housekeeping genes are recognized by RNAP with the primary sigma factor SigA. The primary-like factor SigB is mainly involved in transcription of genes during the transition phase between exponential and stationary growth phases. SigC, SigD, SigE, SigH and SigM of *C. glutamicum* are ECF (extracytoplasmic function) sigma factors involved in responses to various stress conditions (e.g. heat shock, oxidative and surface stresses). To determine, which sigma factors are involved in expression of particular genes, we used the developed *in vitro* transcription system for *C. glutamicum*. Thei *n vitro* transcription system consists of RNAP holoenzyme reconstituted from the purified His-tagged core RNAP and a separately isolated sigma factor. Plasmid pRLG770 constructs carrying promoters of various classes served as templates. Using the *in vitro* analysis, RNAP+SigA recognized specifically the housekeeping promoters P*per* from the *C. glutamicum* plasmid pGA1 and P*veg* from *Bacillus subtilis*, whereas no transcriptional activity of RNAP+SigA on the SigH-dependent P*dnaK* promoter was observed. On the other hand, RNAP+SigH recognized specifically SigH-dependent promoters P*dnaK* and P*sigB* but not the housekeeping promoters. Efficiency of the *in vitro* transcription system was optimized using various concentrations of RNAP and various ratios RNAP/sigma factor. Analyses of further promoters recognized by isolated sigma factors SigB, SigE and SigM are in progress.

RSP002

Identification and characterization of the LysR-type transcriptional regulator HsdR for steroid-inducible expression of the 3α-Hydroxysteroid dehydrogenase/carbonyl reductase gene in Comamonas testosteroni

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3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) from Comamonas testosteroni (C. testosteroni) is a key enzyme in steroid degradation in soil and water. 3α-HSD/CR gene (hsdA) expression can be induced by steroids like testosterone and progesterone. Previously, we have shown that induction of hsdA expression by steroids is a derepression where steroidal inducers bind to two repressors, RepA and RepB, thereby preventing blocking of hsdA transcription and translation, respectively. In the present study, a new LysR-type transcriptional factor HsdR for 3α-HSD/CR expression in C. testosteroni has been identified. The hsdR gene locates 2.58 kb downstream from hsdA on the C. testosteroni ATCC 11996 chromosome with an orientation opposite to hsdA. The hsdR gene was cloned and recombinant HsdR protein was produced, as well as anti-HsdR polyclonal antibodies. While heterologous transformation systems revealed that HsdR activates the expression of hsdA gene, electrophoresis mobility shift assays (EMSA) showed that HsdR specifically binds to the hsdA promoter region. Interestingly, the activity of HsdR is dependent on decreased repression by RepA. Furthermore, in vitro binding assays indicated that HsdR can contact with RNA polymerase. As expected, an hsdR knock-out mutant expressed low levels of 3α-HSD/CR compared to wild type C. testosteroni after testosterone induction. In conclusion, HsdR is a positive transcription factor for the hsdA gene and promote induction of 3α-HSD/CR expression in C. testosteroni.

RSP003

The transcriptional regulatory network of *Corynebacterium jeikeium* K411 and its interaction with fatty acid degradation pathways

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Corynebacterium jeikeium is a natural resident of the human skin and is nowadays frequently recognized as nosocomial pathogen in medical facilities. It causes severe infections in immunocompromised patients and the treatment is often complicated by the comprehensive antibiotic resistance of the organism[1,2]. The most prominent feature of *C. jeikeium* is its lipophilic lifestyle originating from the lack of a fatty acid synthase gene. Fatty acids are essential building blocks for cellular metabolites and membrane or mycolic acid biosynthesis, since *C. jeikeium* is unable to grow solely on other carbon sources such as glucose or acetate[2]. Therefore, the organism encodes a large set of genes involved in β-oxidation, whereof most enzymatic functions relevant for the degradation of fatty acids are encoded by several paralogs[2]. Additionally, C. jeikeium encodes a unique gene cluster that is potentially linked to fatty acid degradation[3].
To understand the transcriptional control of the essential pathway of β-oxidation, the transcriptional regulatory network of the axilla isolate *C. jeikeium* K411 was reconstructed from the complete genome sequence. The current network reconstruction comprises 48 transcriptional regulators and 674 gene-regulatory interactions that can be assigned to five interconnected functional modules. The analyses revealed that most genes involved in lipid degradation are under the combined control of the global cAMP-sensing transcriptional regulator GlxR and the LuxR-family regulator RamA, probably reflecting the essential role of lipid degradation in *C. jeikeium*.

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RSP004

Heterogeneity and timing in autoinducer regulated processes of *Vibrio harveyi*

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Bacteria produce and excrete signaling molecules, so called autoinducers (AIs), which allow them to monitor their population density and/or their environment in a process best known as quorum sensing (QS). The marine bacterium *Vibrio harveyi* uses QS to regulate pathogenicity, biofilm formation, and bioluminescence. The bacterium synthesizes and responds to three different classes of AIs, an acyl-homoserine lactone (HAI-1), a furanosylborate diester (AI-2) and a long-chain ketone (CAI-1).
In order to understand how single cells behave within an AI activated community, AI induced processes were investigated in a homogeneous environment over time. Analysis of wild type single cells revealed that even at high cell densities only 70% of the cells of a population produced bioluminescence. Moreover, fractionation of the population was found for two other AI controlled promoters of genes encoding virulence factors. These results indicate phenotypic heterogeneity of a genetic homogeneous population. An artificial increase of the AI concentrations in the wild type resulted in an all-bright population similarly to a *luxO* deletion mutant, which is AI independent. The capability of this mutant to produce biofilm was significantly reduced. These data suggest that the non-differentiating bacterium *V. harveyi* takes advantage of division of labor.
In addition, results are provided for the temporal variation of the extracellular AI concentrations over time. AI concentrations and QS regulated functions of *V. harveyi* were monitored simultaneously in a growing culture. In the early exponential growth phase only AI-2 was detectable and bioluminescence was induced. In the exponential growth phase both HAI-1 and AI-2 reached their maximum values, bioluminescence further increased and exoproteolytic activity was induced. In the stationary growth phase HAI-1 and AI-2 were adjusted to equal concentrations, exoproteolytic activity reached its maximum, and CAI-1 was detectable. Furthermore, formation of a stable and mature biofilm was dependent on a correct timing of HAI-1 and AI-2 concentrations. Our results demonstrate that not the cell density *per se* is important, but that AIs rather control the development of a *V. harveyi* population.

RSP005

Role of the small RNA RSs2430 in the regulation of photosynthesis genes in *Rhodobacter sphaeroides*

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Small RNAs (sRNAs) play a regulatory role in the adaptation of various bacteria to changing environmental conditions. The identification of sRNAs, using RNA-seq based on 454 pyrosequencing, in the phototrophic bacterium *Rhodobacter sphaeroides* (1) was of major interest because of its high metabolic versatility. In particular, synthesis of the photosynthetic apparatus is regulated in an oxygen- and light-dependent manner. In a physiological screen the sRNA RSs2430 was also found to be influenced by the oxygen tension. Induction of RSs2430 depends on the PrrB/PrrA system, which is a major regulatory system for redox control of photosynthesis genes. Here we present how overexpression and knock

down of RSs2430 influences the expression of photosynthesis genes in *Rhodobacter sphaeroides*.
Northern blots showed that RSs2430 is processed, whereby different 3'ends are generated. The different 3'ends were identified by 3'RACE. Interestingly, only the processed RSs2430-fragments, not the primary transcript, were enriched in the overexpression strain. By using real time RT-PCR and microarray analyses we showed that overexpression of RSs2430 results in a decreased expression of photosynthesis genes.
To study the interaction of RSs2430 and its target mRNAs, a *lacZ* based in vivo reporter system was used. We observed specific translation repression of a light-independent protochlorophyllide reductase subunit N (*bchN*) under high and low oxygen growth conditions.
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RSP006
Examination of a timing mechanism in *Rhodobacter sphaeroides*
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Timing mechanisms are known for over 250 years in eukaryotes. Until now amongst prokaryotes only cyanobacteria could be shown to possess a system to measure time. In *Synechococcus elongatus* a circadian clock builds upon an oscillator of three proteins, KaiA, KaiB and KaiC. A phosphorylation of KaiC in a circadian manner could be shown *in vitro* [1]. All three proteins are essential for clock function. Accordingly, most cyanobacteria possess at least one copy of each gene. An exception is the marine cyanobacterium *Prochlorococcus marinus*, which has suffered a stepwise deletion of the *kaiA* gene [2] but retains a 24 hour rhythm in DNA replication, which is strongly synchronized by alternation of day and night cycles. Surprisingly, the facultative phototrophic proteobacterium *Rhodobacter sphaeroides* possesses a cluster of *kaiBC* genes similar to *Prochlorococcus*. Therefore it has been hypothesized that *R. sphaeroides* may exhibit a rhythmic behavior in gene expression. Such a rhythm has been reported earlier via a luciferase reporter gene system [3]. By microarray analysis, we were able to show a decrease in the expression of photosynthesis genes in a continuously growing *R. sphaeroides* culture after 12 hours of illumination with white light. Preveniently this culture had been put under a 12 hour light/dark rhythm for two days. This data suggests an adaptation to a returning environmental cycle and the existence of a functional timing mechanism in purple photosynthetic bacteria. Furthermore, by an *in vitro* phosphorylation assay an autokinase activity for RspKaiC could be shown which is not altered by the presence of RspKaiB. Future results may shed some light on the existence and evolution of clock systems and circadian rhythms in prokaryotes other than cyanobacteria.
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RSP007
Role of the Irr protein in the regulation of iron metabolism in *Rhodobacter sphaeroides*
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Iron is an essential element for all living organisms. However, since iron potentiates oxygen toxicity by the production of hydroxyl radicals in the Fenton reaction, life in the presence of oxygen requires a strict regulation of iron metabolism.
The Fur family of proteins are well analyzed proteins that regulate transcription of genes in response to iron availability in bacteria (1). In alpha-proteobacteria little is known about the iron mediated gene regulation. The available experimental data suggest that iron regulation mainly occurs by regulators different from Fur (2). The Irr (iron response regulator) protein and its orthologues form a distinct sub-branch of the Fur superfamily but occur only in members of the *Rhizobiales* and *Rhodobacterales* and few other genera. Most iron-dependent genes in alpha-proteobacteria are regulated positively or negatively by Irr (3). At high iron concentration Irr is degraded. ROS seem to promote this degradation indicating another link between iron metabolism and oxidative stress (4). We studied the role of the Irr homologue RSP_3179 in the photosynthetic alpha-proteobacterium *Rhodobacter sphaeroides*.
While Irr had little effect on growth under iron-limiting or non-limiting conditions its deletion resulted in increased resistance to hydrogen peroxide and singlet oxygen. This correlates with an elevated expression of *katE* for catalase in the Irr mutant compared to the wild type under non-stress conditions. Transcriptome studies revealed that Irr strongly affects the expression of genes for iron metabolism, but also has some influence on genes involved in stress responses, citric acid cycle, oxidative phosphorylation, transport, and photosynthesis. Most genes showed higher expression levels in the wild type than in the mutant under normal growth conditions indicating an activator function of Irr. Irr was however not required to activate genes of the iron metabolism in response to iron limitation. This was also true for genes *mbfA* and *ccpA*, which were verified as direct targets of Irr.
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RSP008
Anaerobic toluene metabolism: First evidences for two subtypes of benzylsuccinate synthase
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The aromatic hydrocarbon toluene can be degraded in the absence of oxygen by various facultative or obligate anaerobic bacteria using nitrate, sulphate or Fe(III) as terminal electron acceptor. In all tested strains so far, toluene is activated by an addition reaction of the toluene methyl group to the double bond of fumarate to form benzylsuccinate. This reaction is catalyzed by the benzylsuccinate synthase (Bss), which is a member of the glycyl radical family of enzymes. Even if the overall reaction, catalysed by the Bss, is in all tested strains the same, the alignment of available Bss gene sequences reveals that they slightly differ. This result leads to a possibility to distinguish between several Bss isoenzymes on a genetic level.
In previous *in vivo* studies, data from two dimensional compound specific stable isotope analyses (2D-CSIA) indicated that the reaction mechanism of Bss subtypes in facultative and obligate anaerobes may differ. To test whether the observed isotope fractionation effects are directly due to the Bss reaction mechanisms, *in vitro* assays were performed using cell-free extracts of different facultative and obligate anaerobic toluene degraders. In addition, the enzymatically mediated exchange of hydrogen atoms between toluene and the solvent was investigated. The results of both approaches confirmed the hypothesis that at least two mechanistically different subtypes of Bss exist: one occurring in facultative anaerobes and one occurring in obligate anaerobes. Thus, 2D-CSIA may allow specifically detecting toluene degradation by facultative or obligate anaerobes at contaminated field sites.

RSP009
Characterization of AHL-lactonases and their influence on the quorum sensing system of *Vibrio harveyi*
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Bacteria use signaling molecules, so called autoinducers (AIs) to communicate and to monitor their environment. The marine γ-proteobacterium *Vibrio harveyi* uses three different classes of AIs for communication, HAI-1, a N-(3-hydroxybutyryl)-D-homoserine lactone (AHL), AI-2, a furanosylborate diester and CAI-1, a (*Z*)-3-aminoundec-2-en-4-on. Thereby type III secretion, siderophore production, exoproteolytic activity, biofilm formation, and bioluminescence are regulated.
Heterogeneous behavior of the wild type population with respect to bioluminescence was shown before (Anetzberger *et al*., 2009). The addition of an excess of exogenous AIs resulted in a homogeneous population. It is suggested that the population is able to tightly control the extracellular AI concentrations. *V. harveyi* has five genes encoding putative lactonases.
The putative lactonase VIBHAR_02708, which is highly conserved among *Vibrio* species, was purified and characterized. UPLC coupled MS analysis of the HAI-1 cleavage products confirmed that VIBHAR_02708 is a lactonase. Subsequently, the corresponding deletion mutant was constructed and characterized. The HAI-1 concentration in the culture fluid was about 30% higher in the ΔVIBHAR_02708 mutant than in the wild type. These data clearly show an influence of the lactonase VIBHAR_02708 on the QS system of *V. harveyi* via the adjustment of the HAI-1 concentration.
Anetzberger, C., Pirch, T. and Jung, K. (2009), Heterogeneity in quorum sensing-regulated bioluminescence of *Vibrio harveyi*. Molecular Microbiology, 73: 267-277.

RSP010
Targeted proteome analysis of *Corynebacterium glutamicum*
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The Gram positive soil bacterium *C. glutamicum* is a widely used host organism in industrial biotechnology [1]. Main products are the amino acids L-glutamate, L-lysine and L-threonine. New desired products include building blocks for chemical industry, biofuels and heterologous proteins.

Through intensive investigations aiming at increased production, *C. glutamicum* has become a model organism for systems biology as well [2]. We will present a targeted approach for direct quantification of key enzymes from the central carbon metabolism in *C. glutamicum* raw extracts by high performance liquid chromatography coupled tandem mass spectrometry [LC-MS/MS, 3]. Focusing on glycolysis, TCA, anaplerosis and glyoxylate shunt our method provides a quantitative overview of the enzymes building the core metabolic pathways in *C. glutamicum*. A metabolic labeling strategy with the stable nitrogen isotope ^{15}N is used to overcome measurement errors originating from sample handling and tryptic digestion of protein extracts by isotope dilution mass spectrometry [IDMS, 4].
Sampling batch cultivations of *C. glutamicum* in microtiter plates, our study comprises proteome adaptations to different growth phases and alternative carbon sources. Results show massive reconstitutions of protein levels well agreeing to known changes of metabolic fluxes. Furthermore, we conducted time resolved measurements of protein expression after metabolic switch from glycolytic to gluconeogenetic carbon sources under industrial relevant conditions in stirred tank reactors. Significant changes in protein levels could be detected within 15 min after substrate pulse.
In conclusion we will present a rapid and reliable methodology for quantitative analysis of protein expression and dynamics providing new insights into metabolic regulation of *C. glutamicum*.

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RSP011

Unusual reactions involved in cyclohexanecarboxylate formation during crotonate fermentation in *Syntrophus aciditrophicus*

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The obligately anaerobic Deltaproteobacterium *Syntrophus aciditrophicus* can feed on crotonate as its sole carbon and electron source without a syntrophic partner. The main products of the fermentation pathway are acetate and cyclohexanecarboxylate [1]. The reducing equivalents formed during crotonate oxidation to acetate are recycled by concomitant reduction of crotonate in reverse β-oxidation-like reactions of the benzoyl-CoA degradation pathway. The transiently formed benzoyl-CoA is believed to serve as electron acceptor for recycling redox equivalents yielding six-electron reduced cyclohexanecarboxyl-CoA. We demonstrate that disproportionation reactions of cyclohexa-1,5-diene-1-carboxyl-CoA (1,5-dienoyl-CoA) and cyclohex-1-ene-1-carboxyl-CoA (1-monoenoyl-CoA) are involved in benzoyl-CoA and cyclohexanecarboxyl-CoA formation. These reactions are most likely catalyzed by tungsten containing class II benzoyl-CoA reductases [2]. The cyclohexanecarboxyl -CoA is converted into the end product cyclohexanecarboxylate by a thioesterase or a CoA transferase. The endergonic reductive dearomatization of benzoyl-CoA to 1,5-dienoyl-CoA by NADH ($\Delta G^{\circ\prime}$ = +58 kJ mol^{-1}) can be explained by an electron bifurcation mechanism. We propose that this reaction is driven by the concomitant reduction of 1,5-dienoyl-CoA to 1-monoenoyl-CoA and/or 1-monoenoyl-CoA to cyclohexanecarboxyl-CoA by NADH to ($\Delta G^{\circ\prime}$ < -50 kJ mol-1).

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RSP012

Mutational analysis of the transcriptional regulator AlsR of *Bacillus subtilis*

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Acetoin formation in *Bacillus subtilis* requires acetolactate synthase and -decarboxylase encoded by the *alsSD* operon. The *alsSD* expression is activated in response to fermentative growth conditions, addition of acetate, low pH in the growth medium and aerobic stationary growth. The transcriptional regulator AlsR is essential for *alsS-lacZ* reporter gene expression under all growth conditions tested. The AlsR regulator is a member of the LysR-type transcriptional regulators (LTTR) and composed of two domains: an N-terminal DNA binding domain with a winged HTH motif and a C-terminal regulatory domain which is involved in co-inducer binding and oligomerization.
To identify functional relevant amino acid residues for effector-binding and oligomerization we mutagenized the *alsR* gene in the C-terminal regulatory domain and tested the activity of the produced AlsR mutant proteins in an *in vivo* complementation system. Here, mutated *alsR* genes were integrated into the *amyE* locus of a *B. subtilis alsR* knock out mutant strain and expressed under the control of the xylose-inducible *xylA* promoter. AlsR activity was monitored by ß-galactosidase activities derived from an AlsR-dependent *alsS-lacZ* reporter gene fusion. Several AlsR mutants tested showed reduced *alsS-lacZ* expression *in vivo*.
In addition, we produced and purified the AlsR mutant proteins as Trx/Strep-AlsR fusion proteins and after cleavage with the HRV-3C protease we finally obtained pure AlsR protein. We analyzed the *in vitro* binding ability by EMSA analyses and performed *in vitro* transcription studies with the purified AlsR mutant proteins. The amino acid exchange from serine at position 100 of AlsR to alanine inactivated the AlsR protein for transcriptional activation *in vivo* and *in vitro*. Compared to the wild type protein, the AlsRS100A mutant protein has a defect in DNA-protein complex formation. Whereas, wild type AlsR formed 3 different migrating complexes, AlsRS100A is no longer able to form the slowest migrating complex III in EMSA analyses. Therefore, we deduced complex III as the transcriptional active form. A model of transcriptional active complex formation of AlsR is given.

RSP013

Interconnectivity between two histidine kinase / response regulator systems in *Escherichia coli*

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Bacteria use two-component systems (TCSs) to encounter fluctuating environmental conditions. A membrane-bound histidine kinase (HK) senses a stimulus and transduces it into a cellular signal via phosphorylation. The transfer of this phosphoryl group to a response regulator (RR) with DNA-binding properties mediates the inert reaction, generally an alteration in gene expression (1). Based on the limited number of TCSs in *Escherichia coli* (30/32 HK/RR) it is necessary to coordinate cellular adaptions in order to respond to a multitude of environmental signals. To this end many so called auxiliary proteins have been described recently (2). These proteins can be involved in sensing, scaffolding or connecting TCSs and evolved to an emerging field of bacterial signal transduction.
Although many TCSs in *Escherichia coli* are well characterized, the YehU/YehT and YpdA/YpdB TCSs are largely unknown. Both belong to the group of LytS/LytR-like TCSs comprising of a HK with GAF-domain and a RR with LytTR-DNA-binding domain. Based on bioinformatical data these two TCSs share an amino acid identity of more than 30%. They are wide-spread and co-occure in many γ-proteobacteria (3).
The characterization of the YehU/YehT and the YpdA/YpdB systems revealed reversed transcriptional effects on target genes. Using the bacterial adenylate cyclase-based two-hybrid system YehS was uncovered as hub connecting the two TCSs via protein-protein interactions. Surface plasmon resonance measurements with purified YehS and the RRs confirmed the interactions and suggest an interconnectivity between YehU/YehT and YpdA/YpdB.

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RSP014

Identification of *Marinobacter adhaerens* HP15 genes required for the interaction with the diatom *Thalassosira weissflogii* by In vivo expression technology

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Aggregate formation by living cells and organic matter in the ocean is an important mechanism that mediates sinking of organic carbon. Diatom-bacteria interactions play an important role during this process by inducing secretion of different extra-cellular polysaccharides, which increase the size of marine aggregates. To study cell-to-cell diatom-bacteria interactions, a bilateralin vitromodel system has been established consisting of the diatom *Thalassosira weissflogii* and the marine bacterium *Marinobacter adhaerens* HP15. The bacterium was previously shown to specifically attach to *T. weissflogii* cells, to induce transparent exopolymeric particle formation, and to increase aggregation. In addition, it has been shown that *M. adhaerens* HP15 is genetically accessible, its genome has been sequenced, and several bacterial genes potentially important during the interaction are currently being investigated. However, genes specifically expressedin vivoare still unknown. The aim of this work was to establish anIn Vivo Expression Technology (IVET) screening to identify bacterial genes specifically induced when *M. adhaerens* HP15 interacts with *T. weissflogii*. The IVET vector was constructed by cloning the full-size promoterlesslacZ gene downstream of a promoterless pyrB gene, which encodes an essential growth factor fundamental for pyrimidines biosynthesis. A site-directed mutagenesis approach was used to generate apyrB-deficient mutant in *M. adhaerens* HP15. This mutant was unable to grow in the absence of uracil and in presence of the diatom,

demonstrating its suitability as a selection marker. The introduction of a functional promoter of *M. adhaerens* HP15 into the IVET vector and its subsequent transformation intopyrB-deficient mutant allowed its complementation. Transformants expressing the pyrB gene and lacZ grew in absence of uracil indicating that the system wass functional. The standardization of the IVET screening is currently being tested. Promising genes obtained will be cloned, mutagenized, and characterized in terms of their role in diatom-bacteria interaction. Results of this study will contribute to a better understanding of the molecular mechanisms of diatom-bacteria interactions.

RSP015

Functional analyses of small RNAs in *Agrobacterium tumefaciens*

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Over the last decade, sRNAs have been recognized as widespread regulators of gene expression in bacteria (1). The largest and most extensively studied set of sRNAs act through base pairing with target RNAs, usually modulating the translation and stability of mRNAs (2).
Using a comparative bioinformatic approach (3) we identified diverse sRNAs in the plant pathogen *Agrobacterium tumefaciens*. One sRNA, called AbcR1, controls the expression of at least three ABC transporters among them the periplasmic binding protein of the GABA transporter. It is the first described bacterial sRNA that controls uptake of a plant-generated signaling molecule (4). The molecular details of the sRNA-mRNA interaction will be presented.
By using a differential RNA sequencing (dRNA-seq) technology, we discovered many new sRNA on all four *A. tumefaciens* replicons, the circular chromosome, the linear chromosome, the At-plasmid and the Ti-plasmid (5). Northern blot analyses revealed that several sRNAs were differentially expressed in response to different growth conditions. One sRNA from the Ti-plasmid was massively induced under virulence conditions. Experiments to identify targets of selected sRNAs are under way.

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RSP016

Signal transduction in the thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius*

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Signal transduction from extracellular stimuli to the inside and within the cell is essential for survival of microorganisms. In this process protein kinases and phosphatases often play a key-role and are found in all three domains of life. These enzymes catalyze one of the most important posttranslational modifications, the reversible phosphorylation and dephosphorylation of proteins. Whereas in many Euryarchaeota both, potential histidine kinases and Ser/Thr/Tyr kinases, were found, in the Crenarchaeota just the latter are present.
To date, the knowledge about signal transduction pathways, the inducing conditions and involved proteins in the Crenarchaeota is rather scarce. Therefore, we want to investigate the processes of signal transduction in *Sulfolobus acidocaldarius*. The advantage of this important crenarchaeal model organism is the availability of various genetic tools to performin vivoandin vitrostudies. These tools were used to examine autophosphorylation of some protein kinases and phosphorylation of potential interaction partners. Experimental investigations revealed a connection between motility via the archaeal flagellum and different signal transduction proteins in *S. acidocaldarius*. These results underline the importance of protein phosphorylation in cellular processes of the Archaea.

RSP017

NreA, the third component of the three-component system NreABC of *Staphylococcus carnosus*

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In the facultative anaerobic *Staphylococcus carnosus* the NreABC three component system is required for initiation of nitrate respiration [1]. NreA, NreB, and NreC are encoded within one operon (*nreABC*). The two-component system NreBC is involved in O_2 sensing. NreB acts as a direct oxygen sensor, and the regulator NreC induces the expression of *narGHJI* encoding nitrate reductase under anaerobic conditions [1].
Oxygen sensing by NreB is based on the conversion of the $[4Fe\text{-}4S]^{2+}$ cluster to a $[2Fe\text{-}2S]^{2+}$ cluster by O_2 followed by complete degradation and formation of FeS-less apoNreB [2].
The function of the third component, NreA, was analyzed. NreA is a GAF domain protein. Deletion of NreA leads to a permanent activation of nitrate respiration.
Single-point mutants in NreA were obtained with either loss of nitrate induction, or aerobic derepression, suggesting that NreA controls NreBC function in response to oxygen and nitrate availability.

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RSP018

Binding properties of the transcriptional regulator AlsR of *Bacillus subtilis*

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The transcriptional regulator AlsR is essential for *alsSD* expression in *Bacillus subtilis*. The *alsSD* expression is activated in response to fermentative growth conditions, addition of acetate, low pH in the growth medium and aerobic stationary growth. The *alsSD* operon encodes the acetolactate synthase and -decarboxylase catalysing the production of acetoin from pyruvate. The AlsR regulator is a member of the LysR-type transcriptional regulators (LTTR) composed of two domains: an N-terminal DNA binding domain with a winged HTH motif and a C-terminal regulatory domain which is involved in co-inducer binding and oligomerization.
We analyzed the relevance of single amino acid residues of the DNA-binding domain by site directed mutagenesis and *in vivo* functional analysis of produced AlsR mutant proteins in an *in vivo* complementation system. Here, mutated *alsR* genes were integrated into the *amyE* locus of an *B. subtilis alsR* knock out mutant strain and expressed under the control of the xylose-inducible *xylA* promoter. AlsR activity was monitored by ß-galactosidase activities derived from an AlsR-dependent *alsS-lacZ* reporter gene fusion. Several AlsR mutants tested showed reduced *alsS-lacZ* expression *in vivo*. In addition, we produced and purified the AlsR mutant proteins as AlsR-Strep fusion proteins and analyzed their *in vitro* binding ability by gel retardation analyses.
Using DNase I footprint analyses AlsR binding regions were identified in the *alsS* promoter. A detailed analysis of the DNA sequence revealed several potential palindromic binding sites containing a $T\text{-}N_{11}\text{-}A$ core motif typical for LTTR proteins. To identify the DNA sequences necessary for AlsR binding we changed several TA bases within the proposed AlsR binding region to GG. For this purpose a p-86*alsS-lacZ* reporter gene fusion with 86 bp promoter sequences upstream the transcriptional start site were used. The ß-galactosidase activities mediated by those mutant promoters were determined and compared to the activity of *B. subtilis* carrying the wild type *alsS-lacZ* fusion. In order to directly relate the results of the *in vivo* tested mutated promoter to AlsR binding, we also employed gel retardation assays.

RSP019

The LuxR solo PluR of *Photorhabdus luminescens* senses PLAI-1, a novel endogenous signaling molecule

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Cell-to-cell communication via acyl-homoserine lactones (AHL) is well studied in many Gram-negative bacteria. The prototypical communication system consists of a LuxI-type autoinducer synthase and a LuxR-type receptor that detects the endogenously produced signal. The symbiotic and entomopathogenic enteric bacterium *Photorhabdus luminescens* harbors the plenty of 39 LuxR-like receptors, but lacks any LuxI-type autoinducer synthase and is unable to produce AHL. Here we show that one of these LuxR solos, Plu4562 (PluR), detects an endogenously produced signaling molecule (PLAI-1) that is not an AHL, but a 2-pyrone derivative. We tested different 2-pyrones for induction of *plu4568*-promoter activity, and showed that a novel class of 2-pyrones named photopyrones is produced by different *Photorhabdus* species are the specific signal for PluR. Hence a signaling function for the chemical widespread group of pyrones was identified for the first time for *P. luminescens*. Via PluR, expression of the *plu4568-plu4563* operon is activated, which encodes a putative synthesis pathway correlated with cell clumping. Expression of the *plu4568-plu4563* operon induced cell clumping in *P. luminescens* by addition of PLAI-1 as well as in *E. coli* when induced heterologously. PLAI-1-dependent cell-to-cell communication and the resulting cell clumping seem to be important for colonization of the nematodes by *P. luminescens*.

RSP020

Regulation of anaerobic aromatic hydrocarbons degradation in *Aromatoleum aromaticum* under anaerobic growth condition

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The denitrifying Betaproteobacterium *Aromatoleum aromaticum* utilizes a wide range of aromatic compounds under anoxic condition, among them the hydrocarbons ethylbenzene or toluene. The genes coding for the enzymes of anaerobic toluene metabolism are induced coordinately in the presence of toluene, whereas those coding for the enzymes of anaerobic ethylbenzene metabolism are induced sequentially in the presence of ethylbenzene and the intermediate acetophenone, respectively. Three operons coding for two-component regulatory systems were identified in the genome sequence of *A.aromaticum* as possible candidates for affecting the induction of all toluene-catabolic genes (*tdiSR*) and the induction of ethylbenzene-catabolic genes by ethylbenzene (*ediSR*) and the intermediate acetophenone (*adiRS*). We show here that the (*adiRS*) operon is indeed involved in the acetophenone-dependent regulation of gene expression. The function of these gene products was investigated by genetic and biochemical studies: *adiSR* deletion mutant of *A. aromaticum* was unable to grow on either ethylbenzene or acetophenone and was complemented by adding the *adiRS* genes. Moreover, the predicted acetophenone-sensing histidine kinase (AdiS) was overproduced in *E. coli* and its biochemical properties, i.e. ligand binding, are in line with its proposed function.

[1]-Heider, J., and G. Fuchs.1997. Anaerobic metabolism of aromatic compounds. Eur J Biochem243:577-96
[2]-R. Rabus, M. Kube, A. Beck,,F. Widdel and R. Reinhardt. Genes involved in the anaerobic degradation of ethylbenzene in a denitrifying bacterium, strain EbN1. Arch Microbiol (2002) 178:506-516

RSP021

The W-/Se-containing class II benzoyl-CoA reductase complex in obligately anaerobic bacteria

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Benzoyl-Coenzyme A (CoA) is a central intermediate in the anaerobic degradation of aromatic compounds and serves as substrate for benzoyl-CoA reductases (BCRs). There are two completely different classes of BCRs which both yield the nonaromatic product cyclohexa-1,5-diene-1-carbonyl-CoA [1,2]. Class I BCRs of facultative anaerobes, referred to as BcrABCD, are ATP-dependent, [4Fe-4S] clusters containing enzymes. In contrast, strictly anaerobic bacteria are proposed to employ a W-/Zn-/FeS-/Flavin-/Se-containing, ATP-independent BamBCDEFGHI complex. The active site containing components BamBC were purified and characterized from the aromatic compound degrading model organism *Geobacter metallireducens* [1]. The remaining BamDEFGHI subunits are considered to be involved in the ATP-independent electron activation reaction. We provide evidence that class II BCRs are composed of the predicted high molecular BamBCDEFGHI complex. Initial data indicate that the electron transfer to the aromatic ring is driven by an electron bifurcation process.

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(2) Löffler et al. (2011) Environ Microbiol 13(3) : 696-709

RSP022

Metabolome and transcriptome analysis of *P. aeruginosa* in a chronic lung infection model

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Pseudomonas aeruginosais an ubiquitous environmental soil bacterium and an opportunistic pathogen of humans, animals and plants. It causes chronic infections in patients with cystic fibrosis (CF), chronic obstructive pulmonary disease and bronchiectasis.
We studied the control of virulence factor production depending on metabolic pathways and the transcriptomic state of the organism to understand the activation of specific virulence programs of *P. aeruginosa*. We analysed the metabolome and transcriptome of *P. aeruginosa* in various media and growth phases.
P. aeruginosa PA14 is an acute infection clinical isolate obtained from a burnwound of a patient. It displays pathogenicity in a variety of genetically tractable model hosts and mice.
P. aeruginosa RN7 is a clone of PA14 strain. It is a CF-isolate, which was isolated short after the infection of a patient. RN7 causes chronic disease in experiments with mice.
P. aeruginosa TBCF10839 is a highly virulent strain, which belongs to a major clone in the P. aeruginosa population. It is a pilin-deficient strain that produces large amounts of alginate and shows high resistance against phagocytosis. Being a strong producer of virulence effector proteins, it causes substantial airway pathology in mice after intratracheal instillation.
In an integrative approach of both data sets will be combined to reveal a holistic picture of the adaptive pathway regulation of *P. aeruginosa* in a lung infection and identificate key determinants for the chronic colonization of the human lung.

RSP023

The importance of the GAF domain for K^+-sensing in the sensor kinase KdpD in *Escherichia coli*

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Potassium is the most abundant cation in bacteria and important for different cellular functions. The high affinity K^+ transporter KdpFABC of *E. coli* assures the uptake of K^+ when it is limited in the environment. The production of KdpFABC is regulated by the two-component system KdpD/KdpE, which comprises the membrane-integrated histidine kinase KdpD and the soluble response regulator KdpE. KdpD specifically phosphorylates and dephosphorylates KdpE and therefore regulates the activation and termination of *kdpFABC* transcription, respectively [1]. K^+ has an inhibitory effect on the kinase activity of KdpD *in vitro*, but a K^+-binding site is yet unknown. The kinase activity is also inhibited by Rb^+, but not by Cs^+. New bioinformatic methods revealed that KdpD contains a GAF domain in the C-terminal cytoplasmic region. GAF domains are prominent ligand binding sites and were first identified in c**G**MP-specific cyclic nucleotide phosphodiesterase, **a**denylyl cyclase and the transcription factor **F**hlA. The replacement of the GAF domain of KdpD with the GAF domain of a conserved protein 3e0Y of *Geobacter sulfurreducens* led to a KdpD variant, which caused *kdpFABC* transcription independent of the extracellular K^+ concentration. Hence this KdpD variant was unable to sense K^+. By using site-directed and random mutagenesis three amino acids were identified - two inside and one outside of the GAF domain - which might form a K^+-binding site.

[1] Heermann and Jung, FEMS Microbiol Lett. 2010 Mar; 304(2):97-106.

RSP024

The histidine kinase SgmT is a c-di-GMP receptor and regulates synthesis of an extracellular matrix protease

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Myxococcus xanthus cells are covered by an extracellular matrix composed of exopolysaccharides and proteins, which is indispensable for type pili-dependent motility and fruiting body formation in response to starvation. The orphan DNA binding response regulator DigR plays a role in the regulation of extracellular matrix composition. Using a two-tiered strategy, we genetically and biochemically identify the orphan hybrid histidine kinase SgmT, which contains an N-terminal GAF domain and a C-terminal GGDEF domain, as the partner kinase of DigR. By EMSA and DNase I footprinting experiments, we identify the DigR binding site in the promoter of the *fibA* gene, which encodes a metalloprotease and is the most abundant protein in the extracellular matrix. Whole-genome expression profiling experiments in combination with the identified DigR binding site allowed the identification of candidate members of the DigR regulon and suggest that SgmT/DigR regulate the expression of genes coding for secreted proteins of unknown function, FibA as well as enzymes involved in secondary metabolite synthesis. Our data demonstrate that the N-terminal GAF domain is the primary sensor domain in SgmT and that the C-terminal GGDEF domain binds the second messenger bis-(3'-5')-dimeric cyclic-GMP (c-di-GMP) *in vitro* and functions as a c-di-GMP receptor *in vivo* to spatially sequester SgmT upon c-di-GMP binding. We suggest that SgmT activity is regulated by two sensor domains, the GAF domain and the GGDEF domain, and that binding of ligand to the GAF domain results in SgmT activation and binding of c-di-GMP to the GGDEF domain results in spatial sequestration of SgmT insulating the SgmT/DigR from cross-talk from other signalling systems.

RSP025

The pH-responsive transcriptional activator CadC and its lysine-dependent co-sensor LysP in *E. coli*: New insights into regulatory interplay and signal transduction

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CadC belongs to the ToxR-like transcriptional activators. This type of regulators is characterized by a N-terminal cytoplasmic DNA-binding effector domain and a C-terminal periplasmic sensor domain separated by a single transmembrane helix. ToxR-like proteins combine sensory function and DNA-binding activities in a single polypeptide and lack sites for chemical modification. CadC regulates the expression of the *cadBA* operon and induces transcription under conditions of low external pH and concomitantly available lysine. This in turn allows *E. coli* to adapt to acidic stress. Recently, it was demonstrated that the periplasmic domain of CadC is responsible for pH sensing [1,2]. However, almost nothing is known about the mechanism of signal transduction across the membrane to the cytoplasmic effector domain of CadC. Secondary structure analysis revealed a large unstructured cytosolic loop of unknown function between the transmembrane helix and the DNA-binding domain. To investigate the role of the loop in terms of signal transduction, it was gradually truncated or elongated. Resulting CadC-variants activated *cadBA* expression independent of external pH, implicating that the cytoplasmic loop plays an important role in transducing the signal to the DNA-binding domain. The pH sensor CadC is not a direct lysine sensor, but senses exogenous lysine via an interplay with the lysine transporter LysP. Random and directed mutagenesis was performed to find LysP/CadC interaction sites and to investigate the interconnectivity of the two functions, regulation and transport, of the trigger transporter LysP. This approach led to the identification of LysP variants, which evoke altered interaction patterns with CadC and/or defects in transport activity. Further, transmembrane interactions between LysP and CadC were analyzed in vivo using a bacterial two hybrid (BACTH) system. The BACTH-study provided evidence for a constitutive interaction between sensor and trigger transporter. It is proposed that lysine-induced conformational changes of LysP upon lysine binding and/or transport transduce the external lysine signal to CadC by weakening the persisting protein-protein interaction.

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2. Haneburger I., Eichinger A., Skerra A. and Jung K., J. Biol. Chem. 286 (2011), p. 10681-1068

RSP026

Copper homeostasis in *Corynebacterium glutamicum*

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Copper ions can easily alternate between an oxidised (Cu^{2+}) and a reduced state (Cu^{+}) and are therefore used as cofactor of enzymes involved in electron transport or redox reactions, such as cytochrome *c* oxidases or multicopper oxidases. As high concentrations of uncomplexed copper ions can become toxic for the cell by generating reactive oxygen species or by sulfhydryl depletion, the intracellular concentration of copper must be tightly regulated to prevent toxic levels on the one hand and copper starvation on the other hand. In prokaryotes several copper resistance systems have been identified and characterised, but only little is known about the response to copper starvation conditions.

Here, we have investigated the influence of elevated copper concentrations as well as of copper starvation conditions on *Corynebacterium glutamicum*, a non-pathogenic Gram-positive soil bacterium, which serves as model organism for closely related pathogenic species such as *Mycobacterium tuberculosis*. To gain first insights into copper homeostasis, growth and global gene expression were followed in the presence of different copper concentrations (0 - 500 μM). In this way, the copper excess and the copper starvation stimulon were determined. These and subsequent experiments revealed that the recognition of and response to elevated extracellular copper concentrations is mediated by the two-component system CopRS[1], whereas a one-component transcriptional regulator is responsible for adaption to increased intracellular copper concentrations[2]. Growth of the wild type is only hardly affected under copper starvation, whereas a deletion strain lacking the copper-independent cytochrome *bd* oxidase (Δ*cydAB*) of the branched respiratory chain of *C. glutamicum*[3] exhibits a strong growth defect. The Δ*cydAB* mutant is strictly dependent on the copper-dependent cytochrome aa_3 oxidase, which presumably is at least partially dysfunctional under copper starvation conditions. The phenotype of the Δ*cydAB* mutant and the induction of the *cydAB* genes under copper starvation show that cytochrome *bd* oxidase plays an important role in electron transfer from menaquinol to oxygen under these conditions.

[1] Schelder *et al*., 2011
[2] Schelder *et al*., to be submitted
[3] Bott and Niebisch, 2003

RSP027

Generating a "Gene Knock-out through Allelic Exchange" in *Clostridium acetobutylicum*

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Clostridium acetobutylicum is an anaerobic, Gram-positive soil bacterium and possesses a characteristic biphasic fermentation metabolism. Growing exponentially, the sugar substrates are metabolised to acetate and butyrate, and during the late exponential and stationary phase these acids are converted into the solvents acetone and butanol. The aim of our work is the construction of artificially controlled genes required for solvent production/regulation. So, within the "COSMIC2 SysMO-project" several genes in *C. acetobutylicum* are planned to be deleted by the new "knock-out system".

Aldehyde ferredoxin oxidoreductase (AOR) (CA_C2018) is an oxygen-sensitive enzyme that catalyses the oxidation of aldehydes to their corresponding acids.

Previous transcriptome studies for microarray shift experiments showed an upregulation of several genes of solventogenic and acidogenic metabolism, among them the CA_C2018-gene, during shift from pH 6.5 to pH 5.5. A deletion of this *aor* gene in *C. acetobutylicum* was constructed.

The "allelic exchange system" based on a homologous recombination was used to knock-out the *aor* gene [1]. First, a knock-out cassette was generated and then ligated into the pseudo-suicide vector pMTL-SC7515. After methylation in *Escherichia coli* pANS1 the vector was transformed into *C. acetobutylicum*. After two independent homologous recombinations a double-crossover *C. acetobutylicum*-mutant was established. In further experiments, a complementation of theaorgene will be created. Also, an overexpression mutant for this gene of *C. acetobutylicum* will be generated.

[1] J.T. Heap, O.J. Pennington, S.T. Cartman, N.P. Minton. 2009. A modular system for Clostridium shuttle plasmids. Journal of Microbiological Methods 78: 79-85.

RSP028

Characterization of radical SAM enzymes involved in the heme biosynthesis pathway in *Methanosarcina barkeri*

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The cyclic tetrapyrrole heme is an important cofactor for almost all living organisms. The heme biosynthetic pathway is partly conserved among the three kingdoms. Thus, in eukaryotes, bacteria and archaea 5-aminolevulinic acid serves as the first common precursor for heme biosynthesis. The first cyclic tetrapyrrole of the pathway is uroporphyrinogen III which is converted into coproporphyrinogen III in eukaryotes and most bacteria to generate heme *via* three further steps. In contrast, in archaea and some bacteria an alternative heme biosynthesis pathway is operative in which uroporphyrinogen III is converted into precorrin-2 which is then further transformed into heme [1], [2]. Recently, it was shown that in sulfate-reducing bacteria such as *Desulfovibrio desulfuricans* the alternative route to heme proceeds *via* siroheme, 12,18-didecarboxysiroheme and iron-coproporphyrin III [3]. The enzymes catalyzing the respective transformations of the intermediates represent homologs to enzymes involved in heme d_1 biosynthesis and are also found in heme producing archaea such as the methanogen *Methanosarcina barkeri*.

Our studies are focused on the two heme biosynthesis enzymes NirJ1 and NirJ2 from *M. barkeri* which catalyze the last steps of the alternative heme biosynthesis pathway, namely the removal of two acetate side chains from 12,18-didecarboxysiroheme to form iron-coproporphyrin III (NirJ2) and the subsequent formation of vinyl-groups at tetrapyrrole positions C3 and C8 to synthesize heme (NirJ1). Both, NirJ1 and NirJ2, belong to the radical SAM enzyme family. They contain a CxxxCxxC amino acid motif which is typical for members of the radical SAM superfamily and thus contain a [4Fe-4S] cluster. Additionally, both proteins exhibit a cysteine-rich C-terminus which might harbour a second iron-sulfur cluster involved in catalysis or substrate binding. Strikingly, HemN, the enzyme that catalyzes the vinyl-group formation during the classical heme biosynthesis pathway in bacteria, is also a radical SAM enzyme but contains only a single [4Fe-4S] cluster. Therefore, NirJ1 and HemN catalyze an identical reaction but use different substrates and distinct mechanisms.

[1] T. Ishida, L. Yu, H. Akutsu *et al.* (1998) A primitive pathway of porphyrin biosynthesis and enzymology in *Desulfovibrio vulgaris*. *Proc Natl Acad of Sci USA* **95**, 4853-4858.
[2] B. Buchenau, J. Kahnt, I. U. Heinemann, D. Jahn, and R. K. Thauer (2006) Heme biosynthesis in *Methanosarcina barkeri via* a pathway involving two methylation reactions. *J Bacteriol* **188**, 8666-8668.
[3] S. Bali, A. D. Lawrence *et al.*(2011) Molecular hijacking of siroheme for the synthesis of heme and d_1 heme. *Proc Natl Acad Sci USA* Early Edition.

RSP029

Mutational Analysis within the Periplasmic PAS Domain of the *Escherichia coli* Sensor Kinase DcuS

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E. coli utilizes C_4-dicarboxylates as a carbon source or as an electron acceptor under aerobic and anaerobic conditions, respectively. Metabolic regulation is effected by the two-component system DcuSR, consisting of the membrane-embedded sensor histidine kinase DcuS and the response regulator DcuR. Sensing of C_4-dicarboxylates generates a signal that leads to an autophosphorylation of a conserved histidine residue in the kinase domain of DcuS.
Studying the periplasmic PAS domain (PAS_P) of DcuS revealed both, ON-mutants with a distinct fumarate-independent expression of a *dcuB-lacZ* reporter gene fusion and OFF-mutants showing a considerable loss of DcuS-activation by the effector. ON- and OFF-mutations located outside the binding pocket, were studied in more detail. For these definded sites the chemical and sterical requirements were probed by site-directed mutagenesis, introducing variable residues at one and the same position. In addition the impact of ON- and OFF-mutations on oligomerization and subcelluar localization were tested, using the bacterial two-hybrid system (BACTH) and *in vivo* fluorescence microscopy.
[1] Zientz *et al*. (1998) J Bacteriol 178(24):7241-7247
[2] Scheu *et al*. (2010) J Bacteriol 192(13):3474-3483

RSP030

The transmembrane domain of the sensory histidine kinase DcuS: role in dimerization

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Bacteria are capable of adjusting to changing environmental conditions. To ensure quick adaptation among various conditions, sensors detect stimuli and regulators trigger the cellular response. Two-component systems are widespread in bacteria, consisting of a sensory histidine kinase and a response regulator. The DcuS/DcuR two-component regulatory system of *Escherichia coli* senses C_4-dicarboxylates and stimulates the expression of genes for anaerobic fumarate respiration [1]. The oligomeric state of the sensor DcuS is supposed to be an important parameter for its function [2]. Transmembrane domains (TMDs) of membrane proteins have crucial roles as interaction sites. Dimerization sites in the TMDs of DcuS were analyzed. A tandem SxxxGxxxG motif was identified in TMD2 of DcuS. A combination of bacterial two-hybrid system (BACTH) and GALLEX [3] interaction studies with DcuS variants suggest a role of the GxxxG motif and the TMD in the dimerization of DcuS.
(1) Zientz E., Bongaerts J., Unden G. (1998) J. Bacteriol 180: 5421-5425
(2) Scheu PD, Liao YF, Bauer J, Kneuper H, Basché T, Unden G, Erker W. (2010) J Bacteriol. 192(13):3474-83.
(3) Schneider D, Engelman DM. (2003) J Biol Chem. 31;278(5):3105-11.

RSP031

HtrA-mediated control of nitrate/nitrite assimilation in *S. coelicolor*

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Streptomyces usually grow in nutritional limiting environment often lacking essential elements for growth. *Streptomyces coelicolor*, a model organism for studying the regulation of nitrogen metabolism exhibits a specific regulatory network. In this control system, OmpR-like transcriptional regulation GlnR plays a central role by controlling the transcription of at least 14 genes 9 of which are directly implicated in nitrogen assimilation. During this study, we identified a new GlnR target gene *SCO2958* named *htrA*. *In silico* analysis revealed the presence of two distinct domains in HtrA sequence: an *N*-terminal uroporphyrinogen-III synthase (HemD)-like enzymatic domain and a C-terminal DNA binding domain. Complementation experiments with a hemin auxotroph *E. coli*Δ*hemD* mutant strain showed that HtrA has no HemD activity. Physiological studies of a *S. coelicolor htrA::Tn5062* mutant showed that HtrA is involved in regulation of nitrite reduction. By electrophoretic mobility shift assays the functionality of the HtrA DNA binding domain was confirmed and found that HtrA binds in front of the genes *narK* (putative nitrate extrusion protein), *nirB* (nitrite reductase), *nirA* (putative nitrite/sulphite reductase), and *nasC* (putative nitrate reductase), which are associated with nitrate/nitrite assimilation. Furthermore, a cooperative binding of HtrA together with GlnR to the *nirB* promoter was observed, suggesting that HtrA may act as a "GlnR-helper protein".

RSP032

Posttranslational modification of global response regulator GlnR links nitrogen metabolism and transcription of GlnR target genes in *S. coelicolor*

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Transcriptional regulation of nitrogen assimilation genes inStreptomycescoelicoloris mediated by a global response regulator GlnR. GlnR was shown to act as both a transcriptional repressor and activator underN-limiting conditions. The GlnR-induced genes includeglnAandglnIIwhich encode key enzymes of ammonium assimilation: glutamine synthetases GSI and GSII, respectively. GlnR represses expression of thegdhAgene encoding the glutamate dehydrogenase GDH, which is able to assimilate ammonium into glutamate only under conditions of high ammonium concentrations. Therefore a repression under nitrogen-limiting conditions is reasonable. Finally, GlnR controls reactions for the uptake of ammonium and the utilization other nitrogen sources like nitrate/nitrite or urea. GlnR regulates transcription of the amtB operon,nirBDencoding a nitrite reductase andureAencoding a urease [1]. The mechanism for this repressor/activator function of GlnR was unknown and itself regulation was not investigated till now. We were able to show how the nitrogen status of the cell is connected to the control ofglnRexpression and GlnR activity. Western blot analyses provided evidence that GlnR undergoes posttranslational modification viaSer/Thrphosphorylation andLysacetylation inS. coelicolorM145. LC-MS/MS analyses revealed that underN-excess four serine residues and three threonineresidues were phosphorylated. Additionally two lysine residues were acetylated. The pattern of the modification underN-limited conditions differed significantly (no acetylation and only two phosphorylated serine residues). This kind of regulation is surprising and somehow unusual since GlnR belongs to OmpR-like family and as might be expected it should interact with till now unknown cognate histidine kinase. Various acetylation and phosphorylation patterns influence GlnR´s DNA binding activity. Acetylation seems to completely abolish the binding of GlnR to promoter regions of its target genes. Regulation via acetylation seems to be depending on concentration of nitrogen source however phosphorylation is fine-tuning regulation and depends on type of theN-source. To our knowledge this is the first report aboutLysacetylation andSer /Thrphosphorylation of the response regulator in actinobacteria.
1. Tiffert, Y., Supra, P., Wurm, R., Wohlleben, W., Wagner, R.,Reuther, J., Mol.Microbiol.67(2008) p.861-880

RSP033

Quorum sensing in *Pseudomonas putida* colonies under flow conditions

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Bacterial communication via release and sensing of signal molecules (autoinducer, AI) has been mainly investigated in batch cultures. Here usually coordinated response of the whole population is induced in a cell density dependent manner (quorum sensing, QS). However, most bacteria live heterogeneously distributed in aggregates or biofilms attached to surfaces. Under these conditions, functionality of the signalling system is less well understood and more difficult to approach experimentally. We thus use a combined experimental/mathematical modelling strategy to investigate the induction dynamics of the PpuI/R QS system in *Pseudomonas putida* IsoF. Induction of AI controlled expression of agfpgene was followed with high spatio-temporal (single cell or colony level) resolution. The influence of flow respectively addition of external AI was examined. Main results were: Mass transfer (flow) delays the induction behaviour, probably by removal of AIs. A compartmentation of yet unkown origin occurs, limiting the influence of AI from outside the colony. AI regulation promoted intra- as well as intercolonial heterogeneity. Summarized, there were fundamental differences between the AI functionality in cell aggregates and planktonic batch cultures, which have been analysed before [1]. These differences have consequences for the ecological functionality of autoinducers.
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RSP034

An essential role for cyclic dinucleotide signaling in *Bacillus subtilis*

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Cyclic dinucleotides (c-di-AMP and c-di-GMP) act as second messengers in several bacterial species. In the last decade these messengers have

attracted the attention of molecular microbiologists and there have been several approaches to uncover their signaling landscape.
Bacillus subtilis encodes three putative diadenylate cyclases. One of them, DisA, checks DNA integrity to control cell division and sporulation. In contrast, the function of the other proteins, CdaA and CdaS, is still unknown. Moreover, *B. subtilis* encodes two diguanylate cyclases, CdgA and CdgB. In many bacteria, c-di-GMP governs the lifestyle switch between biofilm formation and motility. However, nothing is known about the function of c-di-GMP in *B. subtilis*.
We have studied the role of these enzymes in the metabolism of cyclic dinucleotides in *B. subtilis*. Both signaling molecules have a tremendous effect on the production of flagellin (Hag), thus they are involved in the control of motility. In contrast, cyclic dinucleotides have no effect on biofilm formation. This similarity of the phenotypes of the mutants has never been observed before and suggests a link between the signaling pathways of the two cyclic signaling molecules.
Another unprecedented result of our studies is the essential function of *B. subtilis* for the formation of c-di-AMP. The genes encoding the two vegetative diadenylate cyclases, DisA and CdaA, could be deleted only when the sporulation-specific enzyme CdaS was artificially expressed in the logarithmic phase. Thus, our results indicate a key role for cyclic dinucleotide signaling in *B. subtilis*.

RSP035
Role of alternative sigma factor PP4553 in stress response and biofilm formation ofPseudomonas putidaKT2440

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Pseudomonas putidais a Gram-negative, aerobic, flagellated and non-pathogenic soil bacterium, which is well known for its extremely metabolic versatility. Because of this, P. putidaoffers a considerable potential for biotechnological applications. The remarkable versatility of this bacterium is at least in parts driven by sophisticated and coordinated regulation of gene expression mediated by a repertoire of transcriptional regulators, in particular the so called sigma factors. Sigma factors are essential for prokaryotic transcription initiation and enable specific binding of the RNA polymerase to the respective promoter recognition sites. Bacteria generally contain one housekeeping sigma factor and a pool of alternative sigma factors which are activated in response to different and often stressful conditions. The genome of P. putida exhibits with 24 a striking number of putative sigma factors, one of which is open reading frame PP4553. To analyze this putative sigma factor in more detail, we constructed a gene knock-out deletion mutant of PP4553 in P. putida KT2440. Further characterization of this PP4553-mutant revealed a twofold increase in attachment as well as biofilm formation on abiotic surfaces in comparison to the wild type strain. Moreover, growth analyses of wild type and PP4553-mutant strain under different stressful conditions suggested that PP4553 is also involved in stress response ofP. putida KT2440. To gain a deeper insight into the regulatory circuit of the putative sigma factor PP4553, we performed transcriptome analysis using Illumina sequencing.

RSP036
Cloning and heterologous expression of naphthoate-CoA ligase from the sulphate-reducing culture N47

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The anaerobic metabolism of naphthalene by the sulphate-reducing culture N47 is initiated by carboxylation to 2- naphtoic acid. N47 is an enrichment culture composed mainly of one member of the deltaProteobacteria. In cell extracts of this culture we have been able to measure a specific 2-naphthoate-CoA-ligase activity which is responsible for the activation of the carboxyl group with HS-CoA. Blasting the amino acid sequence of benzoate-CoA ligase of Rhodopseudomonas palustris[1] against the N47 genome were identified 9 putative 2-naphthoate-CoA ligase candidates. Here, we aim at the purification and characterization of the N47 2-naphthoate-CoA-ligase. The putative genes will be cloned and expressed inE. coli. Primers were designed to remove the native stop codon and to place the gene of interest in frame with an N-terminal His-tag of the expression vector. The gene will be amplified and transferred into an expression system. Functional naphthoate-CoA ligase shall be purified via the His-tag and characterized.

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RSP037
The sensor kinase PA4398 of *Pseudomonas aeruginosa* PA14 regulates swarming motility and biofilm formation

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Multicellular behavior is an important process central to the pathogenesis of *P. aeruginosa*. In addition to biofilm formation, swarming motility represents a second surface-associated community behavior of this human pathogen. Recently, we have shown that swarming can be considered as a distinct physiological state with a tailored metabolic lifestyle or a complex adaptation of *P. aeruginosa* in response to a viscous environment (arguably similar to the mucus rich CF lung) leading to increased antibiotic resistance and virulence gene expression. During an extensive mutant library screening for swarming deficient mutants, we identified a two-component sensor kinase transposon mutant (PA4398) in *P. aeruginosa* PA14 with defects in the ability to swarm on semisolid surfaces (1). To analyze the function of this sensor kinase in more detail, we constructed a knock-out deletion mutant of PA4398 in *P. aeruginosa* PA14 and phenotypically characterized this sensor kinase mutant. In addition to its swarming defect, this mutant also exhibited a decreased production of biofilm mass in comparison to wildtype cells after 24 hours of growth. In contrast, no differences regarding growth rate, twitching and swimming motility were observed. First preliminary microarray analysis revealed the involvement of this sensor kinase in the regulation of nitrogen and iron metabolism.

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RSP038
Will not be presented!

RSP039
Exploring subtilin/nisin hybrid-peptides

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Lantibiotics [1], such as subtilin and nisin are gene-encoded antimicrobial active peptides [2]. These peptides are ribosomally synthesized by *Bacillus subtilis* and *Lactococcus lactis*, as inactive prepeptides [1]. The inactive prepeptides undergo an extensive posttranslational modification to generate active peptides [3, 4]. The posttranslational modifications result in the formation of non-proteinogenic amino acids lanthionine and 3-methyllanthionin, as well as didehydroamino acids. Nisin and subtilin have a similar lanthionin ring structure and they differ in 14 amino acids. Despite their structural similarity, they are highly specific for their respective autoinduction system. In the presence of extracellular subtilin or nisin, a two component system consistent of a histidine kinase and a response regulator interacts with the respective lantibiotic. The histidine kinase is autoinduced in a quorum sensing manner and phosphorylates the response regulator, which in turn induces the expression of the lantibiotic structural gene, the genes of the lantibiotic biosynthesis machinery and the self immunity genes[5].
So far the interaction between subtilin or nisin with their corresponding histidine kinases has not been characterized. To identify the specific binding motif of subtilin and nisin, a β-galactosidase based reporter system for lantibiotic autoinduction was constructed. In addition, a plasmid based expression system was created, which enables the heterologous expression of subtilin/nisin hybrid peptides. These hybrid molecules will be used to analyse and optimize the lantibiotic properties with respect to the activity, stability and solubility. Additionally, these molecules will also identify the specific binding motif between the lantibiotic and its histidine kinase by virtue of their specific autoinduction.

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RSP040
The phytochrome regulon of *Pseudomonas aeruginosa*

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Photoreceptors are able to sense light with specific wavelengths. One of the most familiar biliproteins are red/far-red light sensing phytochromes.

First they were discovered in plants, but later were also described in fungi, cyanobacteria and other prokaryotes. In plants, phytochromes control a wide variety of developmental processes, while their function in prokaryotes is widely unknown. Most bacterial phytochromes contain a histdine-kinase domain suggesting that signal transduction occurs via a two-component regulatory system.Pseudomonas aeruginosais one of the first heterotrophic bacteria in which a phytochrome has been identified. With the two genes *bphO* and *bphP P. aeruginosa* possesses the two necessary components to assemble a red-light photoreceptor system:*bphO* codes for the heme oxygenase to generate the chromophore biliverdin IXα and *bphP*,encoding the apo-phytochrome. So far, no corresponding phytochrome response regulator has been identified yet.
bphO and *bphP* form a bicistronic operon whose expression is controlled by the alternative sigma factor RpoS. New analyses provide an additional regulation of *bphP* by the quorum sensing-regulator LasR.This exceptional regulation is currently addressed to study in more detail. To investigate the function of *bphO* and *bphP* chromosomal knock-out mutants were constructed and analysed. However, no significant phenotypical difference between the mutants and wild type were observed. A combination of expression profile experiments and proteome analyses revealed a link to a *bphP*-mediated stress response. The most downregulated genes are used in a genetic screen to identify the corresponding response regulator of BphP to gain further insight into the function of the phytochrome in *P.aeruginosa* and the components of its regulon. In addition some proteome phosphorylation studies will be presented.

RSP041

A heme-based redox sensor in the methanogenic archaeon *Methanosarcina acetivorans*

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Based on a bioinformatics study, the protein MA4561 from *Methanosarcina acetivorans* was originally predicted to be a phytochrome-like protein [1]. Phytochromes are red light sensing photoreceptors with a bound linear tetrapyrrole chromophore at a conserved cysteine residue in either a PAS or a GAF domain. MA4561 consists of two alternating PAS and GAF domains fused to a C-terminal kinase domain.
While we were able to show that recombinantly produced and purified protein does not bind any linear tetrapyrrole chromophores, UV-vis spectroscopy revealed the presence of a heme tetrapyrrole cofactor. In contrast to many other known heme-containing proteins, the heme was found to be covalently bound via one vinyl side chain to cysteine 656 in the second GAF domain. This GAF domain by itself is sufficient for covalent attachment. The heme cofactor is redox active and is able to coordinate carbon monoxide in its reduced state. Interestingly, the redox state of the heme cofactor has a strong influence on autophosphorylation activity. While reduced and CO-bound protein does not autophosphorylate, the oxidized protein gives a strong autophosphorylation signal. Two-dimensional thin-layer chromatography identified serine and tyrosine residues as phosphorylation sites.
Based on its genomic localization, MA4561 is most likely a sensor kinase of a two-component system. The transcriptional regulator MA4560 (MsrG) encoded downstream of MA4561is directly involved in transcriptional activation ofmtsH, which encodes a methyltransferase/corrinoid fusion protein involved in methylsulfide metabolism [2, 3]. On the basis of our results a model in which MA4561 acts as a heme-based redox sensor is presented.

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RSP042

Functional Analysis of Additional Circadian Clock Proteins in Synechocystissp. PCC 6803

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Circadian rhythms, oscillations with approximately 24 h periods driving many physiological activities, are found in most eukaryotes. Among prokaryotes, exclusively cyanobacteria are known to harbour an internal clock. Work on the model strain for the circadian clock, Synechococcus elongatus PCC 7942 has shown that the interaction of only 3 proteins, KaiA, KaiB and KaiC encoded by the kaiABC gene cluster is essential for the generation of circadian rhythms of gene expression. The timing process itself is based on rhythmic changes in the autophosphatase-, autokinase- and ATPase- activity of the hexameric KaiC protein.
A few cyanobacteria show variations among their circadian clock gene composition, such as the loss of kaiA in the case of Prochlorococcus. In contrast, the genome of Synechocystissp. PCC 6803 holds an additional kaiC2B2 operon and two orphan kaiB3 and kaiC3 genes in addition to the kaiABC gene cluster. We are currently investigating the function of these additional kai genes.
Analysis of Synechocystis kai mutants indicates that kaiC2 is an essential gene. Knockdown mutants of the kaiC2B2 operon display a bleached phenotype. Biochemical characterization of purified KaiC2 protein suggests that it possesses kinase activity and might interact with components of the phycobilisome as well as with the transcription machinery. Further biochemical characterization will yield insights into Kai protein complex formation, as well as ATPase activity and phosphorylation cycles of the three different KaiC proteins from Synechocystis.

RSP043

Model of the synthesis of trisporic acid in Mucorales showing bistability

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An important substance in the signaling between individuals of Mucor-like fungi is trisporic acid (TA). This compound, as well as some of its precursors, serves as a pheromone in mating between (+)- and (-)-mating types. Moreover, intermediates of the TA pathway are exchanged between the two mating partners. Here, we present mathematical models of the synthesis pathways of TA in the two mating types of an idealized Mucor-fungus, based on differential equations. These models include the positive feedback of TA on its own synthesis. We compare three sub-models in view of bistability, robustness and the reversibility of transitions. Our modelling study showed that, in a system where intermediates
are exchanged, a reversible transition between the two stable steady states occurs, while an exchange of the end product leads to an irreversible transition. The reversible transition is physiologically favoured, because the high-production state of TA must come to an end eventually. Moreover, the exchange of intermediates and TA is compared with the 3-way handshake widely used by computers linked in a network.

RSP044

Cysteine formation with *Corynebacterium glutamicum* and intracellular sensing of O-acetyl-serine

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We succeeded to engineer *Corynebacterium glutamicum* into a superior L-serine producing microorganism. L-Serine is a precurser of L-cysteine and both amino acids are required for pharmaceutical purposes. Consequently, it is of interest to study the two step conversion of L-serine to L-cysteine mediated by serine acetyltransferase (SAT, *cysE*) and O-acetylserine sulfhydrylase (OASS, *cysK*). The L-cysteine synthesis involves the intermediate O-acetyl-serine (OAS) which is demonstrated to interact *in vitro* with the transcriptional regulator CysR. We fused the CysR target *cysI* to EYFP to construct pSenOAS. Presence of pSenOAS resulted in increased fluorescence of cultures with elevated OAS levels, illustrating that *in vivo* OAS interacts with CysR to cause increased *cysI* transcription. The system established allows the detection of cells with elevated OAS levels at the single cell-level and the differentiation and sorting of single cells according to their cytosolic OAS concentration via FACS (Fluorescence Activated Cell Sorting).
The L-serine producer accumulated already 1 mM L-cysteine. Upon overexpression of *cysE* 5.8 mM L-cysteine accumulated, and upon the combined expression of *cysE* plus *cysK* 7.3 mM L-cysteine was found. The work illustrates that *C. glutamicum* is a promising candidate for the overproduction of L-cysteine, and that FACS selection is a tool for further strain development.

RSP045

Identification of the target promoters of Qdr1 and Qdr2, two transcriptional regulators of 2-methylquinoline degradation by *Arthrobacter nitroguajacolicus* Rü61a

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Arthrobacter nitroguajacolicus Rü61a is a Gram-positive soil bacterium which is able to utilize 2-methylquinoline as source of carbon and energy. The genes that are required for the conversion of 2-methylquinoline to anthranilate are clustered in two divergently oriented “upper pathway” operons (pAL1.003-006 and pAL1.007-011). A third operon (pAL1.019-023) codes for enzymes involved in anthranilate degradation via coenzyme

A (CoA)-thioester intermediates. All three operons are located on the linear 113 kbp plasmid pAL1 [1].
The DNA region comprising the catabolic operons also contains two genes, *qdr1* (quinaldine degradation repressor) (pAL1.016) and *qdr2* (pAL1.024), which code for proteins similar to PaaX, a GntR family transcriptional regulator. This family contains more than 250 members which recognize highly diverse palindromic operator regions [2]. PaaX is the main regulator of the phenylacetate catabolon of *Escherichia coli* [3] and *Pseudomonas putida* [4] and acts as transcriptional repressor in the absence of its specific effector phenylacetyl-CoA.
Electrophoretic mobility shift assays (EMSA) with recombinant Qdr1 and Qdr2 showed that both regulators bind specifically to the promoter regions of the catabolic operons, and revealed that the dissociation of Qdr-DNA complexes is mediated by anthraniloyl-CoA, i.e., a very late intermediate of 2-methylquinoline degradation. Interestingly, Qdr2 also retards the migration of *qdr1* and *qdr2* promoter fragments. Analysis of the promoter region of the operon comprising pAL1.007-011 by EMSA with different competitor DNA fragments enabled us to narrow down the recognition site of Qdr2 to a 40 nt region. However, consensus sequences for PaaX-like or other GntR regulators as reported by Rigali *et al*. [2] were not evident.
The differential roles of Qdr1 and Qdr2 in the regulation of the 2-methylquinoline degradation pathway of *A. nitroguajacolicus* Rü61a are not yet fully understood. Particularly the presumed auto- and/or reciprocal regulation of the *qdr* genes by their own gene products requires further investigations. For this purpose the interactions between Qdr1 and Qdr2 and all promoter regions are currently being studied by EMSA, antibody supershift analysis and exonuclease III footprinting.
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RSP046

The redox sensor Rex controls product formation in *Clostridium acetobutylicum*

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The anaerobic bacterium *Clostridium acetobutylicum* is well known for its biphasic fermentation metabolism. The exponential growth is characterized by acetic and butyric acid formation and during the stationary phase the solvents acetone, butanol and ethanol are the main products. However, very little is known about regulatory and molecular mechanisms controlling the carbon and electron flow during the metabolic shift. The sensing of the redox status of the cell is expected to play an important role within this regulatory network.The genome of *Clostridium acetobutylicum* encodes the protein Cac2713, which is annotated as "redox sensing transcriptional repressor Rex". The deduced amino acid sequence of Rex shows a high similarity to well-known NADH/NAD^+ redox regulators. To analyze the function of Rex in *C. acetobutylicum*, a Rex negative mutant of *C. acetobutylicum* was constructed by insertional inactivation of the gene. The mutant exhibited an interesting phenotype. In batch culture this strain produced high amounts of ethanol and butanol production started earlier at higher pH-value compared to the parental strain. The production of butyric acid and acetone was significantly reduced. In agreement with the physiological data the genes of several dehydrogenases, including the bifunctional aldehyde/alcohol dehydrogenase AdhE2 (Cap0035) were upregulated as shown by Northern blot analysis. Furthermore, the purified Rex protein was able to bind to putative Rex boxes in front of these genes.
We concluded that Rex plays an important role in product formation by sensing the redox status of the cell and adjusting the metabolic flux accordingly.

RSP047

The impact of the stringent response on rRNA transcription in *Staphylococcus aureus*

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The stringent response is a conserved regulatory system present in almost all bacterial species. Nutrient limitation provokes the synthesis of (p)ppGpp. The mechanisms by which these molecules result in the profound reprogramming of the cell physiology are still much debated. The most conserved feature of the stringent control, namely down-regulation of rRNA synthesis, seems to be regulated by fundamentally different mechanisms dependent on the organisms analysed. For *Bacillus subtilis* it was proposed that a lowering of the intracellular GTP pool leads to transcriptional inactivation of the rRNA operons, which are initiated by iGTP. In *Staphylococcus aureus* three (p)ppGpp synthetases (RSH, RelP and RelQ) are present. We have constructed in-frame deletion mutants in *rsh*, *relP* and *relQ* as well as a double and a triple mutant. The (p)ppGpp synthesis provoked by amino acid deprivation is accompanied by a drop of the GTP pool. To analyse rRNA regulation in *S. aureus* we first determined the transcriptional start sites of the *rrn1* operon by RACE (rapid amplification of cDNA ends). The main promoter initiates with an iGTP (P1), the other with an iTTP (P2). For measurement of promoter activity we cloned the single promoters (P1, P2) of the *rrn1* operon in front of a truncated *gfp* gene and integrated these constructs into the chromosome. *Rrn1* transcription was assessed in the WT and in the (p)ppGpp synthetase mutants under different conditions. Analysis of the single promoters revealed that: I) In the WT both the P1 and P2 promoters are clearly down-regulated within 1 h of amino acid deprivation. II) This down-regulation is RSH-dependent, since in the *rsh* mutant the P1 and P2 originating transcripts are even up-regulated under stringent conditions. III) Such an effect was not observed using a control promoter driving the two-component system saeRS and initiating with iATP. Thus, both *rrn1* promoters are specifically down-regulated in a RSH-dependent manner. In conclusion, since only one of them initiates with an iGTP, the lowering of the GTP pool can only partially explain the RSH-dependent down-regulation of rRNA synthesis in the human pathogen *S. aureus*.

RSP048

A deep sequencing approach to identify sRNAs in *Streptomyces coelicolor*

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Latest studies have revealed that bacteria encode a wide range of small noncoding RNAs (sRNAs) and more and more are being discovered. The function of most of these sRNAs is still unclear though they are increasingly recognized as important regulators in bacteria. In the majority of cases they act as antisense riboregulators at the post-transcriptional level. They are usually encoded in the intergenic regions of the genome and their expression pattern is often linked to different points in time during development or to specific stress conditions.
We were interested in sRNAs of *Streptomyces coelicolor*. Streptomycetesare filamentous $Gram^+$ bacteria with a high G+C content which produce a large variety of secondary metabolites, especially antibiotics.
We took an RNomics approach to identify sRNAs in *S. coelicolor*. We isolated total RNA and performed deep sequencing using the 454 technology. RNA was prepared from bacteriagrown in rich media to stationary phase. We obtained 58,000 reads from the sequencing and compared them to the *S. coelicolor* genome. After bioinformatic analysis, we obtained 63 candidates with a length from 82-494 nt. In addition, we were able to detect 192 transcriptional start sites.
We selected 24 interesting candidates, which are located in intergenic regions of the genome and are at least 80 nt in length and highly expressed, for further experiments. The expression of the putative sRNAs was validated by Northern Blot.
We will present data of sRNA candidates which show a growth phase dependent expression. We now intend to identify their targets by analyzing knock down and overexpression mutants.
Vockenhuber MP., Scharma CM., Statt MG., Schmidt D., Xu Z., Dietrich S., Liesegang H., Mathews DH., Suess B. (2011) Deep sequencing-based identification of small non-coding RNAs inStreptomyces coelicolor.RNA Biol.,1; 8(3).

RSP049

The interaction of transcription factor TnrA with glutamine synthetase and PII-like protein GlnK

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TnrA is the major transcription factor in *Bacillus subtilis* that controls gene expression in response to nitrogen availability [Wray *et al*., 2001]. When the preferred nitrogen source is in excess, feedback-inhibited glutamine synthetase (GS) was earlier shown to bind TnrA and disable its activity. During nitrogen-limited growth TnrA is fully membrane bound via an AmtB-GlnK complex [Heinrich *et al*., 2006]. The complete removal of nitrate from the medium leads to rapid degradation of TnrA in wild-type cells. We suppose that binding of TnrA to GlnK or GS is required for both regulation of TnrA activity and its protection from proteolysis.
In the AmtB- or GlnK-deficient strains, TnrA is present in a soluble state in cytoplasm and does not degrade in response to nitrate depletion. We have found that TnrA forms either a stable soluble complex with GlnK in the absence of AmtB or constitutively binds to GS in the absence of GlnK, and is protected thereby from proteolysis. It was shown previously that the TnrA C-terminus is responsible for interactions with (GS) [Wray *et al*., 2007]. To check whether the C-terminus of TnrA is also required for interaction with GlnK, various truncations of N-terminally His_6-tagged TnrA (lacking 6, 20 and 35 amino acids from C-terminus) were

constructed and overexpressed in *E.coli* cells. By pull-down analysis it was established that deletion of already 6 C-terminal amino acids abrogate GS binding. The region between 20 and 35 amino acids from the C-terminus is required for GlnK interaction as well as for proteolysis of TnrA. These data confirm that the interaction of GS or GlnK with TnrA protects it from degradation. Alternatively, if ammonium was added to nitrogen starved cells, TnrA dissociates from GlnK and binds to GS. Interaction of TnrA with GS inactivates the transcription factor. Conversely, TnrA inhibits the GS activity; TnrA represses *in vitro* the biosynthetic activity of GS, independently of the presence of AMP or glutamine.

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RSP050

Cross-interactions between two-component signal transduction systems in *E. coli*

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Microorganisms commonly use 'two-component' signaling systems for sensing environmental conditions. Prototypical two-component systems are comprised of a sensory histidine kinase and a response regulator protein that is phosphorylated by the kinase. The regulator typically acts as a transcription factor regulating gene expression. Apart from a few studies performed *in vitro*, the signaling properties of a whole prokaryotic two-component network *in vivo* remains largely unclear. We use a system level approach to characterize the interactions between sensors, regulators and promotors in the model bacterium *Escherichia coli* on different levels, using *in vivo* fluorescence resonance energy transfer (FRET) microscopy and flow cytometry. We measure a set of labelled sensor dimers and sensor-regulator combinations at physiological expression levels and describe quantitatively their interaction strength and kinetics using FRET. Additionally, we identify mixed complexes between different sensors and non-cognate sensor-regulator pairs exhibiting *in vivo* interactions. These findings indicate possible interconnections between different signaling pathways. We demonstrate that in some of the cases interactions are sensitive to specific stimulation, suggesting that changes in protein arrangement play a role in signal processing. Using flow cytometry and transcriptional reporters, we further observe several cases where sensors have an effect on non-cognate promotor regulation, indicating the physiological relevance of the identified interconnections between different signal transduction pathways. Our results should help to establish an integral picture of cell signalling, which is of general importance for single cellular organisms.

RSP051

SyR1 - a sRNA regulating photosynthesis in cyanobacteria

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Post-transcriptional gene regulation by trans encoded small RNAs (sRNA) emerges as an regulatory feature common to most prokaryotes. Recently, biocomputational prediction [1], comparative transcriptional analysis [2] and high throughput pyrosequencing of Synechocystis sp. PCC6803 [3] revealed the existence of many new sRNAs in this cyanobacterial model organism. One of these candidates is the strongly accumulating sRNA SyR1 (Synechocystis ncRNA 1), which is a 130nt long transcript from the intergenic region between the fabX and hoH genes. More detailed investigation on SyR1 showed that this sRNA is upregulated under high-light stress and CO2 depletion [2] and that a strain overexpressing Syr1 exhibits a bleaching-phenotype lacking photosynthetic pigments. A homology search revealed SyR1 candidates in other cyanobacteria while a bioinformatical target prediction implies that the predominant interaction site, which is also the most conserved sequence element of SyR1, potentially binds to the transcripts of photosynthesis genes. Moreover, gel mobility shift assays provide evidence for a direct interaction between SyR1 and psaL and ongoing mutational analysis of the putative SyR1 binding site aims to verify the post-transcriptional regulation of this target gene. Furthermore, preliminary results indicate that long-term SyR1 overexpression leads to a down-regulation of genes involved in the high-affinity uptake of inorganic carbon (Ci) while the aeration of cultures with 5% CO2 quickly abolishes SyR1 accumulation in the overexpression strain and complements the bleaching-phenotype. For these findings we speculate that SyR1-dependent gene regulation affects photosystem biosynthesis and homeostasis and possibly integrates light and Ci-signaling pathways.

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RSP052

Utilization of metabolic regulation for the production of heterologous proteins in *Burkholderia glumae*

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Burkholderia glumae is a Gram-negative proteobacteria. Although initially proposed to be part of the *Pseudomonas* genus, this strain was transferred along with others like *Pseudomonas cepacia* and *Pseudomonas gladioli* to the new genus *Burkholderia*. Since the rice pathogen *B. glumae* is non-human pathogenic and therefore classified as S1-organism, it could be used as model organism for related pathogenic bacteria like *Pseudomonas aeruginosa*.

Due to its relevancy for agriculture, most of the scientific investigations with regard to *B. glumae* focused on the mechanisms the rice-pathogenicity is based on. Besides, *B. glumae* has an interesting industrial application range: The BASF company has developed *B. glumae* by classical strain improvement as a lipase over-production strain[1,2]. Thus, there is the possibility to produce large amounts of functional enzyme and we want to gain access to this production capacity for heterologous protein production by establishing *B. glumae* as a novel expression strain.

Expression systems based on the T7-Polymerase are able to produce large amounts of proteins, for example in *E. coli*, but lead in some cases to inactive enzymes accumulated in inclusion bodies. Here, posttranslational modification, folding, and secretion of proteins may be crucial steps in successful production of proteins and active enzymes. We want to avoid these problems by inducing the T7-Polymerase expression at a time *B. glumae* is able to handle large amounts of produced proteins, like its lipase. Therefore, we have created an expression strain which exhibits a lipase promoter controlled T7-Polymerase gene. The transcription of genes downstream this lipase promoter can be induced for example by olive oil[3]. Since we have shown that the lipase promoter is controllable and inducible by the choice of additional carbon sources in the culture medium, we have also constructed a vector-based expression system for *B. glumae* containing a lipase promoter. The production capacity and prevention of inclusion bodies for difficult-to-express genes will be determined in further studies.

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RSP053

An expression system for the W-containing class II benzoyl-coenzyme A reductases in *Geobacter metallireducens*

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In anaerobic bacteria most aromatic growth substrates are converted into the central intermediate benzoyl-coenzyme A (benzoyl-CoA). Benzoyl-CoA reductases (BCRs) dearomatize benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA). Obligately anaerobic bacteria such as *Geobacter metallireducens* employ class II benzoyl-CoA reductases. The active site components of this W-enzyme, BamBC, have recently been isolated and characterized[1]. A genetic system comprising a suitable expression plasmid was established in *Geobacter metallireducens* that enabled the active production of Strep-tagged BamB, which supposedly contains tungsten. Surprisingly, the electron transferring wild type BamC subunit, containing 3 [4Fe-4S] clusters, was co-purified with Strep-tagged BamB indicating a strong but reversible interaction of the two subunits. The established system enables the efficient production and purification of class II benzoyl-CoA reductase subunits and may enable expression of other W-/metallo enzymes from obligately anaerobic Deltaproteobacteria.

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RSP054

Insight into the (de)phosphorylation of the phosphotransferase proteins HPr and Crh in *Bacillus subtilis*

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In *Bacillus subtilis* uptake and utilization of different carbon sources are tightly regulated by carbon catabolite repression (CCR) (1). The global players involved in CCR are HPr and the HPr kinase/phosphorylase. Upon phosphorylation of HPr at Ser~46, CCR is mediated by the CcpA-HPr-

Ser46~P complex. Additionally, *B. subtilis* possesses the carbon-flux regulator Crh. Like HPr, Crh can be phoshorylated at the regulatory site Ser~46. However, the influence of Crh on CCR is weak. Recent studies showed that Crh senses the metabolic state of *B. subtilis*, thereby controlling flux through the toxic methylglyoxal pathway (2).
Different carbohydrates form a hierarchy in their ability to trigger phosphorylation of HPr and Crh by HPrK/P. Upon utilization of preferred carbon sources HPr and Crh are predominantly phosphorylated. In contrast, the non-phosphorylated forms prevail in the presence of secondary substrates (3, 4).
Here we are focusing on conditions leading to dephosphorylation of HPr and Crh at Ser~46. Both, Crh and HPr accumulate in their non-phosphorylated forms upon entry in the stationary phase. We demonstrate that phosphorylation as well as dephosphorylation of Crh is carried out by the single enzyme, HPrK/P. In contrast, it turned out that dephosphorylation of HPr depends on a different enzyme, namely a phosphatase of the PP2C family. The physiological consequences of the involvement of this phosphatase are discussed.

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RSP055

Novel structures of PII signal transduction proteins from oxygenic phototropic organisms

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PII proteins constitute one of the most widely distributed families of signal transduction proteins, whose representatives are present in archaea, bacteria and plants. They play a pivotal role to control the nitrogen metabolism in response to the central metabolites ATP, ADP and 2-oxoglutarate (2-OG). These signals from energy status, carbon and nitrogen metabolism are integrated and transmitted to the regulatory targets (key enzymes, transporters and transcription factors). In oxygenic phototrophic organisms, from cyanobacteria to higher plants, the controlling enzyme of arginine synthesis, N-acetyl-glutamate kinase (NAGK), is a major PII target, whose activity responds to the cellular 2-OG and energy status via PII signalling. Novel crystal structures of PII signal transduction proteins from oxygenic phototrophs in the presence of signaling metabolites and in complex with NAGK give deeper insights into their control mechanism and sheds light on the evolutionary adaptation of PII signal transduction.

RSP056

Hierarchy of Selenoprotein Gene Expression in the Archaeon *Methanococcus maripaludis*

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Proteins containing selenocysteine are found in members of all three domains of life, Bacteria, Eukarya and Archaea. A dedicated tRNA ($tRNA^{sec}$) serves as a scaffold for selenocysteine synthesis. However, sequence and secondary structures differ in $tRNA^{sec}$ from the different domains. An Escherichia coli strain lacking the gene for $tRNA^{sec}$ could only be complemented with the homolog from Methanococcus maripaludis when a single base in the anticodon loop was exchanged demonstrating that this base is a crucial determinant for archaeal $tRNA^{sec}$ to function in E. coli. Complementation in trans of M. maripaludis JJ mutants lacking $tRNA^{sec}$, O-phosphoseryl-$tRNA^{sec}$ kinase, or O-phosphoseryl-$tRNA^{sec}$:selenocysteine synthase with the corresponding genes from M. maripaludis S2 restored the mutant's ability to synthesize selenoproteins. However, only partial restoration of the wild-type selenoproteome was observed as only selenocysteine-containing formate dehydrogenase was synthesized. Quantification of transcripts showed that disrupting the pathway of selenocysteine synthesis leads to down-regulation of selenoprotein gene expression, concomitant with up-regulation of a selenium-independent backup system, which is not re-adjusted upon complementation. This transcriptional arrest was independent of selenophosphate but depended on the "history" of the mutants and was inheritable, which suggests that a stable genetic switch may cause the resulting hierarchy of selenoproteins synthesized.

RSV1-FG

Signal recognition and transmission by the CpxAR-two component system

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Two-component systems (TCS) are the predominant signalling systems allowing bacteria to communicate with their environment [1]. In general, a TCS comprises of a sensor kinase (SK) and a response regulator (RR). Upon stimulation the SK is autophosphorylated and transfers the phosphoryl group to the RR which acts now a transcription regulator of target genes. To balance the response some SKs also dephosphorylate the phosphorylated RR. However, the mechanistic details in signal recognition and transmission by TCS are still only poorly understood.
The Cpx-TCS is a well established model ubiquitous in Gram-negative bacteria that integrates a broad variety of different signals including salt stress, pH stress, lipids and misfolded proteins that cause envelope stress [2]. The Cpx-TCS is made up of the SK CpxA, the RR CpxR and the accessory protein CpxP. CpxP is known to shut off the Cpx-TCS by inhibiting CpxA autophosphorylation [3] and to promote degradation of misfolded pilus subunits [4]. Recent structural and functional studies provide first insight into how CpxP inhibits CpxA and serves as sensor for misfolded pilus subunits, pH and salt [5]. Now, we have used membrane-SPINE [6] to demonstrate not only the direct interaction between CpxP and CpxA under non-stress conditions but also the release of CpxP from CpxA under certain stress conditions *in vivo*.
Other signals are CpxP-independent recognized by CpxA as a misfolded variant of the maltose binding protein that activates phophotransfer to CpxR [2] and lipids that inhibit dephosphorylation of activated CpxR. Hence, independent entry points for the Cpx-TCS exist that result in specific activities of CpxA. In addition, we will present a structure homology model of the catalytic domain of CpxA in complex with CpxR that we have proved for critical residues in the interface between both proteins *in vivo*. Thus, we have now the system and methods in hand to gain a deeper understanding of signal recognition and transmission over the membrane in a TCS in general.

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RSV2-FG

Mechanism of signal transfer by the tandem hamp domain from *Natronomonas pharaonis*

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Chemotaxis and phototaxis in bacteria share most steps in signal transducing mechanisms. External stimuli are converted via a HAMP domain (**H**istidine **k**inases, **A**denylyl cyclases [ACs], **M**ethyl-accepting chemotaxis proteins [MCP] and **P**hosphatases) into a conformational response of the output machinery, mostly kinases or kinase control units which impinge on the flagellar motor to affect swimming behaviour. Hitherto, more than 12,000 HAMP domains are annotated in the EMBL data bank; only a few have been functionally characterized (1).
The phototransducer from *Natronomonas pharaonis* (NpHtrII) belongs to the group of MCP´s. It receives its signal from the light excited sensory rhodopsin (SR II) which forms a complex with the transducer Htr II (2). SRII initiates a sliding of the second transmembrane helix of HtrII (3) resulting in a conformational change in the HAMP domain (rotation and translation) which sets off the signalling cascade. HtrII, unlike many of the well studied MCP's has a tandem HAMP domain ($HAMP_{Htr}$I and II).
We study signal conversion through such a tandem HAMP domain by generating chimeras with Tsr, the *E. coli* serine receptor, and the mycobacterial AC Rv3645 as a reporter enzyme. Such chimeras have been shown to be regulated by serine using the HAMP domain of either Tsr or of Rv3645 (4). After sequence comparisons with positively operating HAMP domains two chimeras with mutated Htr II tandem HAMP were generated, which responded differently to the serine signal between sensor and output-domains i.e an inversion of signal was observed. The mechanistic differences in these two chimeras, which lead to completely opposite output, are being actively investigated.

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RSV3-FG

Signalling within resistance modules against peptide antibiotics - regulatory interplay between ABC-transporters and two-component systems

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Over the last decade, a number of ABC-transporters have been identified as resistance determinants against antimicrobial peptides. Their regulation generally occurs via two-component systems (TCSs), which are most commonly encoded in the same genetic loci as the transporters. Both the transport permease and sensor kinase components of these modules show unusual domain architecture: the permeases contain ten transmembrane helices and a large extracellular loop, while the sensor kinases lack any obvious input domain. Strikingly, in these systems the ABC-transporter and TCS have an absolute and mutual requirement for each other in both sensing of and resistance to antimicrobial peptides, suggesting a novel mode of signal transduction in which the transporter constitutes the actual sensor. A search of several non-redundant protein databases revealed the existence of over 250 such systems, predominantly among *Firmicutes* bacteria. Parallel phylogenetic analysis of the permease and sensor kinase components revealed a tight evolutionary correlation, suggesting a functional conservation of their unusual signalling mechanism. Additionally, based on the observed co-clustering, we could identify putative corresponding TCSs for those transporters lacking a regulatory system in their immediate neighbourhood.
To test our predictions experimentally, *Enterococcus faecalis* was chosen as model organism, because it possesses two such ABC-transporters and one TCS, which are located in three separate genetic loci, and co-clustering was observed for the TCS with one of the transporters. Expression of both transporters was induced by the peptide antibiotics bacitracin and mersacidin. Interestingly, the transporter which matched the TCS in the phylogenetic classification was required for regulation, while the second transporter appeared to mediate the actual resistance against the target compounds. In summary, our results show that these types of ABC-transporters and TCSs have co-evolved to form self-sufficient detoxification modules against antimicrobial peptides, and suggest a novel signalling mechanism involving communication between transport permease and histidine kinase. Furthermore, our phylogenetic classification can be applied to the prediction of such regulatory interactions among previously uncharacterized systems.

RSV4-FG

The one-component regulator CadC of *E. coli* is a targetof the elongation factor P

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The bacterial elongation factor P (EF-P) has been extensively investigated for more than 30 years (1,2). The protein is known to stimulate the ribosomal peptidyl transferase activity in vitro (2,3). EF-P is suggested to have a translational effect on a limited number of mRNAs and plays a role in bacterial virulence (4,5). Neither a direct target nor the in vivo function is known yet. Here we report a translational effect of EF-P on the one-component regulatory protein CadC. To maintain internal homeostasis at low external pH CadC activates transcription of the *cadAB* operon encoding the lysine decarboxylase CadA and the lysine/cadaverine antiporter CadB, respectively.
A transposon screen was used to identify genes involved in CadC regulation. One insertion was found in *yjeK* encoding a 2,3-lysine aminomutase. YjeK together with the lysyl-tRNA-synthase YjeA catalyse the post-translational modification of EF-P at position Lys34 (4,6,7). By testing *E. coli* in frame deletions strains of *yjeA*, *yjeK* and *efp* we could show that active EF-P is essential for *cadBA* expression. Moreover, protein levels of CadC were significantly reduced in an *efp* mutant. Subsequently, a series of *cadC-lacZ*-translational reporter fusions strains was constructed. Based on the pattern of ß-galactosidase activities it is suggested that EF-P affects elongation but not initiation of CadC translation. The identification of EF-P as direct translational effector on CadC represents not only a new regulatory principle, but provides new insights into the role of EF-P and its highly conserved eukaryotic ortholog eIF5a.

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RSV5-FG

A Zn^{2+}-sensory diguanylate-cyclase from *Escherichia coli*

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The bacterial second messenger cyclic dimeric GMP (c-di-GMP) is a key factor controlling biofilm formation in many bacterial species. C-di-GMP is produced from two GTP molecules by diguanylate cyclases and degraded by specific phosphodiesterases. Typically, bacterial genomes encode for many of these signaling enzymes and almost all of them harbor signal input-domains of various types at their N-termini. Upon perception of largely unknown physicochemical stimuli the input-domains modulate the enzymatic activity of the output domains and by this influence the intracellular concentration of c-di-GMP.
Here we show that the E. coli diguanylate cyclase YdeH is a *bona* fide high affinity sensor for Zn^{2+}.The crystal structure of the widely conserved Zn^{2+}-bound input domain of YdeH shows coordination of the cation via three completely conserved histidine and one conserved cysteine residue. Zinc removal *in vitro* strongly and reversibly stimulates YdeH-activity. Addition of Zinc to the growth medium downregulates *E. coli* biofilm formation via reduced production of the extracellular matrix component poly-b-1,6-N-acetyl-glucosamine and in a YdeH-dependent manner. Mutation of the Zinc-coordinating amino acids leads to strong and constitutive activation of YdeH by mimicking a Zinc-deprived state and causes hyperbiofilm formation. YdeH represents the first example of a biological zinc-sensor that exerts its downstream effects posttranscriptionally and it is the first example of a metal sensory c-di-GMP signaling protein.

RSV6-FG

Reactive oxygen species-inducible ECF σ factors of *Bradyrhizobium japonicum*

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Extracytoplasmic function (ECF) σ factors control the transcription of genes involved in different features, such as stress responses, metal homeostasis, virulence-related traits, and cell envelope structure. The genome of *Bradyrhizobium japonicum*, the nitrogen-fixing soybean endosymbiont, encodes 17 predicted ECF σ factors. Genes for two of them, bll1028 and blr3038, are highly induced in response to the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). The blr3038 gene, but not bll1028, is associated with the predicted anti-σ factor gene blr3039. Mutants lacking Bll1028, Blr3038 and Blr3039, Blr3039 or both σ factors were constructed and phenotypically characterized. Although host legume plants are known to exert oxidative stress on infecting rhizobia, the mutants are symbiotically proficient when tested on three different host plants (soybean, mungbean, cowpea). In free-living conditions, the mutants are more sensitive to singlet oxygen than the wild type. Potential target genes of Bll1028 and Blr3038 were determined by microarray analyses. These data disclosed that each of the two ECF σ factors controls a distinct, rather small set of genes with about half of them belonging to the much larger regulon of H_2O_2-inducible genes.

SIV1-FG

The regulation of cnidarian-dinoflagellate mutualisms: in sickness and in health

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Cnidarians such as reef-building corals engage in a mutualistic symbiosis with intracellular photosynthetic dinoflagellates. This intimate partnership forms the trophic and structural foundation of coral reef ecosystems. This presentation examine the cellular and molecular mechanisms underlying the establishment, maintenance and breakdown of the symbiosis in coral- and anemone-dinoflagellate partnerships. There is increasing evidence from both genomics and functional studies that host innate immunity and symbiont strategies for modulating this immune response are central to the stability of the symbiosis. During onset of symbiosis these mechanisms include, lectin-glycan signaling, host innate immunity, host cell apoptosis and changes in host membrane trafficking. Coral bleaching, a severe

threat to the health of reefs worldwide, results from the dysfunction and collapse of the symbiosis. Several studies suggest that coral bleaching is a host innate immune response to a symbiont compromised by severe oxidative stress. This evidence includes increased nitric oxide levels, and host cell apoptosis and autophagy in heat-stressed animals, all well-known immune mechanisms in other systems to eliminate detrimental microbial invaders.

SIV2-FG

Amount, activity and mode of transmission of microbial symbionts associated with the Caribbean sponge *Ectyoplasia ferox*

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Many marine sponges contain large amounts of phylogenetically complex yet highly sponge-specific microbial consortia within the mesohyl matrix. While vertical transmission has been shown in various marine sponges [1,2,3], the impact of horizontal/ environmental transmission has not been investigated so far. This study provides insights into vertical and horizontal/ environmental transmission of sponge symbionts using adult, embryonic and larval material of the Caribbean sponge *Ectyoplasia ferox*. Transmission-electron microscopy revealed large amounts of morphologically diverse microorganisms in the adult and embryonic tissue. Counting of DAPI stained bacteria in adult sponge tissue homogenates displayed a loss of 50% of the sponge microorganisms during spawning. By sequencing approximately 250 16S rRNA gene library clones and by using a 99% similarity threshold, OTUs were obtained for adult (44), embryonic (13) and larval (12) sponge material. Denaturing gradient gel electrophoresis (DGGE) showed highly similar banding patterns between the three developmental stages, indicating that sponge specific symbionts are transmitted vertically. Activity profiling by comparing 16S rRNA and 16S rRNA genes via DGGE revealed, that nearly all symbionts are metabolically active in all three developmental stages. Initial attempts to create symbiont-free sponge larvae by the addition of antibiotics were promising. As observed by DGGE, the amount of bacteria inside the larvae could be reduced significantly. However symbiont free sponge larvae were not obtained, likely because of the short incubation time of four days. In summary, it was shown, that the three *E. ferox* developmental stages contained highly similar microbial consortia, which confirms previous observations that sponge-specific microbial consortia are passed on via vertical transmission. These symbionts are furthermore metabolically active in all developmental stages. In addition, the expulsion of up to 50% of sponge symbiont biomass into the environment during spawning and their potential uptake again by other sponges renders horizontal/ environmental transmission at least as another possibility.

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SIV3-FG

Highly specific nematode symbioses from the North Sea and the benefits of harbouring ectosymbionts

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Ectosymbiotic bacteria are widespread on marine organisms but the specificity of these associations and the beneficial role of the symbionts are still poorly understood. Stilbonematid nematodes from sulfidic coastal sediments carry a characteristic coat of sulfur-oxidizing ectosymbionts on their cuticle. It is widely believed that these ectosymbionts provide nutrition to their hosts but no clear evidence has been provided so far. To investigate specificity and the role of ectosymbiotic bacteria we looked at stilbonematid nematodes of the genus *Leptonemella* from intertidal sandy sediments of the North Sea island of Sylt. To date, three co-occuring *Leptonemella* species have been described from Sylt based on their morphology. Our first aim was to investigate the specificity of the *Leptonemella* symbioses by using molecular methods. Phylogenetic analysis based on the 18S rRNA marker gene of the nematodes revealed an unexpectedly high diversity of at least five *Leptonemella* species that are closely related to *Leptonemella* species from the Mediterranean Sea. Clone libraries of the 16S rRNA gene and the ribosomal intergenic spacer region (ITS) of the ectosymbionts showed that these are closely related to the gammaproteobacterial sulfur-oxidizing ectosymbionts of other nematode host species as well as the endosymbionts of gutless marine and oligochaetes (the so-called MONTS clade for Marine Oligochaete and Nematode Symbionts). Remarkably, each of the five host species has its own distinct 16S-ITS rRNA symbiont phylotype, indicating that these ectosymbioses are highly specific, despite the fact that the hosts co-occur and acquire their symbionts from the environment. Our second aim was to test the hypothesis that the ectosymbionts provide their hosts with nutrition. We incubated the worms and their symbionts with radiolabelled bicarbonate and measured inorganic carbon fixation by the symbionts and transfer of fixed carbon to the host. We developed a method to separate the ectosymbionts from the worms so that the radioactive label could be measured in each separately. With this method we showed that the symbionts incorporate radiolabelled carbon, which is then transferred to the host. We are currently using nanoscale secondary ion mass spectrometry (NanoSIMS) on *Leptonemella* tissue sections to examine the transfer of carbon in more detail. Our results show that there is a high degree of specificity in the ectosymbiotic associations of these very closely related co-occurring host species and that the hosts benefit nutritionally from their symbionts.

SIV4-FG

Digesting the diversity - evolutionary patterns in the gut microbiota of termites and cockroaches

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From a phylogenetic viewpoint, termites are a family of social cockroaches. In addition, close relatives of bacterial lineages considered typical for termite intestinal tracts have also been occasionally found in cockroach guts. This gives rise to the hypothesis that elements of the gut microbiota found in different termite lineages are derived from their common evolutionary ancestors. However, the microbial diversity in the guts of every termite family has not been fully explored, and even less is known about the microbiota of cockroach guts. We comprehensively analyzed the bacterial communities in the microbe-packed hindguts of 34 dictyopteran species by amplification of the V3-V4 region of bacterial 16S rRNA genes with a modified primer set and subsequent 454 pyrotag sequencing. The communities were analyzed both on the basis of sequence similarity and according to hierarchical classification. Thorough statistical and community analyses revealed that the cockroach gut microbiota is more diverse and less specialized than that of termites. The bacterial community compositions differed significantly already at the phylum level. Nevertheless, we found a core microbiota of groups of *Lachnospiraceae*, *Synergistaceae*, and other taxa in all insects investigated, which strongly supports the hypothesis that elements of the termite gut microbiota were present already in the common ancestor. A remarkable increase in relative abundance of certain bacterial lineages correlates with the feeding guilds, which indicates that the gut microbiota provides a reservoir of bacterial diversity that is exploited when new functions are required, e.g., for the degradation of particular dietary components. Taken together, the emerging patterns document a long history of (co)evolution between the gut microbiota and their dictyopteran host species, resulting in a clear and distinct clustering of the bacterial communities that reflects both the phylogeny and the feeding guild of their hosts.

SIV5-FG

Metabolic activity of the obligate intracellular amoeba symbiont *Protochlamydia amoebophila* in a host-free environment

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Prior to 1997, chlamydiae were exclusively perceived as pathogens of humans and animals, and our knowledge about their biology was restricted to members of the family *Chlamydiaceae*, including the human pathogens *Chlamydia trachomatis* and *Chlamydia pneumoniae*. Today we know that the true diversity within the phylum *Chlamydiae* is larger than ever thought before. Many of the more recently discovered chlamydiae exist in phylogenetically diverse hosts in the environment. One of the eight currently known chlamydial families, the *Parachlamydiaceae*, is well known to comprise natural symbionts of free-living amoebae. A common feature of all chlamydiae is their obligate intracellular lifestyle which comes along with a unique biphasic developmental cycle. The so called elementary body (EB) constitutes the infective form and was perceived as a spore-like stage which is metabolically inert. However, recent studies challenged this dogma and provided first evidence for an extracellular activity of EBs. The aim of this study was the characterization of the metabolic capabilities of EBs of the amoeba symbiont *Protochlamydia amoebophila*. For this purpose, EBs were purified from their host cells and incubated with isotope-labeled substrates in a host-free environment. Isotope-ratio mass spectrometry (IRMS) and fourier transform ion

cyclotron resonance mass spectrometry (FTICR-MS) provided first insights into the metabolic pathways active in *P. amoebophila* EBs and showed that: (I) *P. amoebophila* EBs take up D-glucose and several amino acids in host free environments and incorporate carbon and nitrogen into their biomass. (II) Host free-incubated *P. amoebophila* EBs release 13CO2 from 13C-D-glucose, which is a clear indication for respiration. (III) Bio-conversion of glucose was observed and suggested synthesis of sugar polymers, which likely serve as storage compounds. (IV) The availability of D-glucose during host-free incubation significantly affects maintenance of infectivity. In summary, our data clearly demonstrate metabolic activity of *P. amoebophila* EBs. Intriguingly, this active metabolism seems to play a key role for maintenance of infectivity and establishment of a symbiotic relationship with its amoeba host.

SIV6-FG

Bacteria-zooplankton interactions: a key to understanding bacterial dynamics and biogeochemical processes in lakes?

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Worldwide, metazoan zooplankton represents an enormous surface and biomass in pelagic systems but their linkage with bacteria has been assumed to be rather indirect (via nutrient cycling and trophic cascades). However, a zooplankter's body carries a high abundance of diverse bacteria, which can account for a substantial fraction and diversity of pelagic bacteria. Zooplankton bodies are organic-rich micro-environments that support fast bacterial growth. Their physical-chemical conditions differ from those in the surrounding water and hence select for different bacterial communities. Until now, information on bacteria-zooplankton interactions is still limited to only a few zooplankton groups and environments, in particular copepods in coastal and estuarine waters. Therefore, our proposal focuses on bacteria-zooplankton interactions in lakes. Since zooplankton taxa can have very different life history traits we will compare a large number of zooplankton taxa in a variety of lakes. In field and lab studies we will investigate these interactions with a high spatial and temporal resolution. We will address 4 topics: A) spatial and temporal variations in bacteria-zooplankton association, B) microbial dynamics in the zooplankton gut microhabitat, C) bacterial dispersal by migrating zooplankton and D) effects on microbial activities during the mid-summer zooplankton decline. We aim to fundamentally change the way we understand pelagic food webs and the ecological role of bacteria-metazoan interactions.

SIV7-FG

Efflux pumps and TetR-like regulators in rhizobial interactions with plants

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Our goal is to analyze the importance of efflux pumps that are being used by plant-associated bacteria to defend themselves against secondary plant metabolites. In Bradyrhizobium *japonicum* and in *Sinorhizobium meliloti*, the nitrogen-fixing symbionts of soybean and alfalfa, respectively, genes encoding efflux pumps of the major facilitator superfamily have been found to be induced by plant flavonoids. Interestingly, adjacent to these genes are genes encoding TetR-like regulators. The respective intergenic regions contain several palindromic structures, presumably binding sites for the TetR-like proteins. Our comparative analysis, concomitantly carried out with *B. japonicum* and *S. meliloti*, characterizes the binding of purified regulator proteins to the operator regions, determines the influence of flavonoids on the binding affinities, analyzes the expression of the efflux pump genes in dependence of flavonoids, and determines the phenotype of bacterial mutants concerning their resistance towards plant-derived compounds and their competitiveness in plant-bacteria interactions. These studies will shed further light on the intricacies of the molecular signal exchange between rhizobia and their legume host plants.

SIV8-FG

Host colonization of bifidobacteria - from genome sequence to protein function

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Bifidobacteria are one of the major bacterial groups of the human colonic microflora and are widely used as probiotics due to their reported health-promoting effects. *Bifidobacterium bifidum* S17, *B. longum* ssp. *infantis* E18 and *B. breve* S27 were shown to have opposing phenotypes regarding adhesion to intestinal epithelial cells (IECs) and anti-inflammatory effects. While *B. bifidum* S17 tightly adheres to cultured IECs and shows prominent anti-inflammatory effects both *in vitro* and in several murine models of colitis, the other two strains show week adhesion and no anti-inflammatory capacity [1, 2].
In order to study these differences in more detail, we sequenced the genomes of these strains [3, unpublished data] and analysed them with a special focus on factors involved in adhesion and host colonization. A large number of proteins were identified in all strains that display domains potentially involved in adhesion to host tissues. All strains possess gene clusters, which show high similarity to genes encoding for pili structures in Gram-positive bacteria, and the corresponding genes are differentially expressed in the tested bifidobacteria under *in vitro* conditions. Comparison to other genome sequences led to the identification of a lipoprotein of the bacterial cell envelope, which is specific for the species *B. bifidum*. Functional analysis revealed that this protein plays an important role in adhesion of *B. bifidum* strains to IECs. Furthermore, a gene encoding a subtilisin-family protease was identified in the genome of *B. bifidum* S17, which might be involved in host colonization and/or probiotic effects. The corresponding gene was cloned and expressed in *E. coli* and purified protein was analysed for its substrate specificity.
Using genome sequencing, comparative analysis and functional characterisation, a number of factors were identified in different strains of bifidobacteria, which could play an important role in host colonization of these important human symbiotic bacteria.

1. J. Preising, D. Philippe, M. Gleinser, H. Wei, S. Blum, B.J. Eikmanns, J.H. Niess, C.U. Riedel. Applied and Environmental Microbiology **76** (2010): 3048-51.
2. D. Philippe, E. Heupel, S. Blum-Sperisen, C.U. Riedel. International Journal of Food Microbiology **149** (2011): 45-9.
3. D. Zhurina, A. Zomer, M. Gleinser, V.F. Brancaccio, M. Auchter, M.S. Waidmann, C. Westermann, D. van Sinderen, C.U. Riedel. Journal of Bacteriology 193 (2011): 301-2.

SIP1-FG

Host species-specific *Thiothrix ectosymbionts* on cave-dwelling amphipods

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Symbioses between invertebrates and chemoautotrophic microbes are common in the marine environment, and ecologically dominant at deep-sea hydrothermal vents, cold seeps, and coastal sediments. The association between *Niphargus ictus* amphipods and *Thiothrix* bacteria, found in the Frasassi caves of central Italy, is the first known example of a chemoautotrophic symbiosis from a freshwater habitat. The Frasassi cave system is forming by sulfuric acid-driven limestone dissolution and hosts an underground ecosystem sustained by chemoautotrophy. Thick mats of filamentous sulfur-oxidizing gamma- and epsilonproteobacteria cover the sulfidic cave water bodies. Gammaridean amphipods of the genus *Niphargus* interact directly with these bacterial mats, but only a specific *Thiothrix* phylotype, which is rarely found in the mats, has been identified on their exoskeletons [1].
When the symbiosis was first described, it was assumed to involve only one host species, *N. ictus*. Subsequent molecular and morphological analyses revealed that there are two other *Niphargus* species in Frasassi, and that the three species have independently invaded the cave ecosystem [2]. Scanning Electron Microscopy (SEM) showed that these two additional species also harbor filamentous bacteria, and their assignment to the sulfur-oxidizing *Thiothrix* clade was confirmed based on their 16S rRNA gene sequences. Phylogenetic analyses and Fluorescence In Situ Hybridization (FISH) revealed that the three *Niphargus* species harbor three different *Thiothrix* symbionts, one of which is specific to one host, and two of which are shared between two hosts. Automated Ribosomal Intergenic Spacer Analyses (ARISA) showed that the distribution of these *Thiothrix* symbionts among *Niphargusis* strongly host species-specific.
The three Niphargusspecies display different locomotive behaviors and occupy distinct microhabitats within the cave system. Consequently, they might expose

their ectosymbionts to varying sulfide and oxygen regimes. Using incubations with ^{13}C-labeled carbon compounds and ^{15}N-labeled nitrogen gas followed by NanoSIMS analyses, we found that symbiont metabolism reflects the geochemical niches provided by the host amphipods.

1. S. Dattagupta et al., ISME J3(2009), 935-943
2. J.-F. Flot, G. Wörheide and S. Dattagupta, BMC Evol Biol10(2010), 171

SIP2-FG

Enrichment of a novel lineage of methanogenic archaea distantly related to the *Thermoplasmatales* from the intestinal tract of termites

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The subdomain Euryarchaeota comprises both methanogenic and non-methanogenic archaea, and several lineages of uncultivated archaea with unknown properties. One of these deep-branching lineages was first discovered in the gut of termites and was shown to be distantly related to the Thermoplasmatales. By comparative phylogenetic analysis, we connected this lineage of 16S rRNA genes to a large clade of unknown sequences of *mcrA* genes, a functional marker for methanogenesis that shows the same tree topology as the 16S rRNA. The evidence for a new order of methanogenic archaea was corroborated by methanogenic enrichment culture from the gut of a *Cubitermes* species, which yielded a single archaeal 16S rRNA gene and a single *mcrA* gene by direct DNA sequencing. The sequence data confirmed the congruence of both lineages in the respective trees. Related sequences were found in the guts of other termites and cockroaches, but are also encountered in the intestinal tracts of mammals and in various environmental samples.

SIP3-FG

Are you cereus? -*Arthromitus* filaments in the guts of arthropods

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Filamentous bacteria attached to the gut wall of many arthropods were first described and collectively named *Arthromitus* by Joseph Leidy more than 160 years ago. Since then their identity has remained contentious. *Arthromitus* was controversially claimed to be a life stage ofBacillus cereusby Lynn Margulis and colleagues based on cultivation attempts. Others have merely assumed that *Arthromitus* belongs to the same lineage as the segmented filamentous bacteria (SFB) of vertebrate guts, the only commensal micro-organisms known to specifically modulate the host immune response. We used single cell manipulation and a full-cycle rRNA approach to show unequivocally that *Arthromitus* belongs neither to *B. cereus* nor is it closely related to the SFB. Instead, *Arthromitus* represents a diverse lineage of exclusively arthropod-associated sequences within the family *Lachnospiraceae*. Based on the distinct taxonomic positions of *Arthromitus* and SFB, we propose to no longer use the provisional name "*Candidatus* Arthromitus" for SFB but to reserve it for the filaments of arthropods originally described by Leidy. Although the function of *Arthromitus* remains unknown, these bacteriaseem to be restricted to termites, cockroaches, scarab beetle larvae, and millipedes - the only terrestrial arthropods that produce methane.

SIP4-FG

The *Bradyrhizobium japonicum* protein NopE1 - a type III-secreted effector protein with self-cleavage activity

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Bradyrhizobium japonicum is a symbiont of soybean and secretes proteins induced by the isoflavone genistein. Two of these type III-secreted proteins are the homologs NopE1 and NopE2, which exhibit 77% sequence identity. In plant experiments, it was shown that the proteins affect nodulation positively or negatively depending on the host [1]. Reporter assays revealed that NopE1 and NopE2 are translocated into the plant cell. Both proteins contain two similar domains of unknown function (DUF1521). NopE1 and truncated derivatives were expressed in *E. coli* as GST fusion proteins and purified with glutathione sepharose affinity chromatography. NopE1 contains an autoproteolytic cleavage site between an aspartate and proline within each of the DUF1521 domains [1]. Self-processing of the protein can be induced by calcium and is not influenced by protease inhibitors that do not complex the calcium ions [2]. Experiments with truncated derivatives show that the minimal domain required for autocleavage is the DUF1521 domain. Under native conditions, NopE1 forms dimers and the fragmented protein parts adhere to each other. Database searches indicate the presence of the DUF1521 domain in proteins from different Proteobacteria, e.g. *Vibrio coralliilyticus* and *Burkholderia phytofirmans*. Therefore, this domain probably serves a function in several non-related interactions between bacteria and their eukaryotic host.

[1] Wenzel et al. (2010). The type III-secreted protein NopE1 affects symbiosis and exhibits a calcium-dependent autocleavage activity. Mol. Plant-Microbe Interact., 23, 124-129.
[2] Schirrmeister et al.(2011) Characterization of NopE1 a self-cleaving nodulation effector protein of *Bradyrhizobium japonicum*. J. Bacteriol., 193(15):3733-3739

SMV001

Will not be presented!

SMV002

Distribution, diversity, and activity of anaerobic ammonium oxidizing bacteria in soils

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Denitrification and anammox, the anaerobic microbiological conversion of ammonium with nitrite (or nitrate) to N_2, are the only processes closing the global nitrogen cycle. Anammox is increasingly recognized as an important process for wastewater treatment and nitrogen cycling in marine ecosystems [1]. Conversely, knowledge about distribution, diversity, and activity of anammox bacteria in the terrestrial realm is only starting to emerge [2]. A variety of soils were tested for the presence of anammox bacteria using standard and quantitative PCR. The diversity of anammox bacteria was assessed by cloning/sequencing of the 16S rRNA gene, and anoxic soil incubations with ^{15}N-labeled substrates were employed to quantify anammox activity.

Anammox bacteria were detect in wetland soils, lakeshores, a contaminated porous aquifer, permafrost soil, marsh sediment, and in soil samples associated with nitrophilic plants. Candidate genera "Brocadia", "Kuenenia", "Scalindua", "Anammoxoglobus", "Jettenia", and sequences of two new clusters were identified, representing a higher genus level diversity than in marine environments where mostly "Scalindua" is found. Changes in the phylogenetic structure of the anammox guild along the soil profile suggest that the different candidate species occupy separate niches. Moreover, anammox bacteria were not present in every tested soil type or soil fraction, demonstrating their heterogeneous distribution and their specific ecological requirements. Abundance and activity of anammox increased with soil depth yet varied little with season. Data show that anammox can be a significant process in certain soils although denitrification remains so far the dominant N_2-eliminating process.

[1] Kuenen J.G. (2008) Nat. Rev. Microbiol. 6:320-326.
[2] Humbert S. et al. (2010) ISME J. 4:450-454.

SMV003

Denitrification activity of a new and diverse denitrifier community in a pH neutral fen soil in Finnish Lapland is nitrate limited

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Wetlands are sources of the greenhouse gas N_2O. Peatlands cover about 25% of the Finnish land area and might significantly impact on N_2O fluxes. Denitrifiers release N_2O as an intermediate. The denitrifier community in a pH-neutral fen (pH app. 6.9) in Finnish Lapland was investigated. N_2O emission was not observed *in situ* from unsupplemented fen soil during gas chamber measurements, but nitrate and ammonium addition significantly increased *in situ* N_2O emissions. Stimulation with nitrate was stronger than with ammonium, indicating denitrification rather than nitrification as a potential source of N_2O *in situ*. N_2O was produced and subsequently consumed in gas chambers, indicating complete denitrifcation to N_2. In unsupplemented anoxic microcosms, fen soil produced N_2O only when acetylene was added to block nitrous oxide reductase, likewise indicating complete denitrification. Nitrate and nitrite stimulated denitrification in fen soil, and maximal reaction velocities (v_{max}) of nitrate or nitrite dependent denitrification where 18 and 52 nmol N_2O h^{-1} g_{DW}^{-1}, respectively. N_2O was below 30% of total produced N gases in fen soil when concentrations of nitrate and nitrite were <500 μM, indicating essentially complete denitrification. Denitrifier diversity was assessed by analyses of *narG*, *nirK/nirS*, and *nosZ* (encoding nitrate-, nitrite-, and nitrous oxide reductases, respectively) by barcoded amplicon pyrosequencing. Analyses of ~28,000 quality filtered sequences indicated up to 26 species-level operational taxonomic units (OTUs), and up to 363 OTUs at 97% sequence similarity, suggesting diverse denitrifiers. Phylogenetic analyses revealed clusters distantly related to publicly available sequences, suggesting hitherto unknown denitrifiers. Representatives of species-level OTUs were affiliated with sequences of unknown soil bacteria and Actinobacterial, Alpha-, Beta-, Gamma-, and Delta-Proteobacterial sequences. Comparison of the 4 gene markers at 97% similarity indicated a higher diversity of *narG* than for the other gene markers based on Shannon indices and observed number of OTUs. The collective data indicate (i) a high denitrification and N_2O consumption

potential, and (ii) a highly diverse, nitrate limted denitrifier community associated with potential N_2O fluxes in a pH-neutral fen soil.

SMV004

Emission of Denitrification-derived Nitrogenous Gases by Brazilian Earthworms

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Earthworms are an abundant soil macrofauna. Small to medium sized earthworms belonging to the family Lumbricidae emit the greenhouse gas nitrous oxide (N_2O) and dinitrogen (N_2) produced by ingested denitrifiying soil bacteria. The large earthworm *Octochaetus multiporus* (Megascolecidae) from New Zealand does not emit nitrogenous gases but its gut displays a high denitrification potential. To extend the knowledge about the emission of nitrogenous gases (i.e., N_2O and N_2) by earthworms, nine small, medium and large earthworm species belonging to the families Glossoscolecidae (*Rhinodrilus alatus*, *Glossoscolex paulistus*, *Glossoscolex* sp., *Pontoscolex corethrurus*), Megascolecidae (*Amynthas gracilis*, *Perionyx excavatus*), Acanthodrilidae (*Dichogaster annae*, *Dichogaster* sp.), and Eudrilidae (*Eudrilus eugeniae*) from Brazil were analyzed. All earthworm species except for *G. paulistus* and *G.* sp. emitted N_2O. Except for *D.* sp., acetylene greatly increased the emission of N_2O indicating denitrification as the main source of N_2O. On a per worm basis, the up to 63 cm long *R. alatus* emitted the highest amounts of nitrogenous gases, primarily N_2 indicative of complete denitrification. Nitrite greatly stimulated the emission of N_2O and N_2 by *A. gracilis* and resulted in a minor emission of N_2O and N_2 by *G. paulistus*. Gut nitrate reducers and denitrifiers of gut content and soil of *G. paulistus* (large) and *A. gracilis* (small) were analyzed via barcoded amplicon pyrosequencing with the structural gene markers *narG*, *nirK*, and *nosZ*, encoding for a subunit of the nitrate reductase, nitrite reductase, and N_2O reductase, respectively. Gene sequences of *narG*, *nirK*, and *nosZ* in the gut and soil of *G. paulistus* were highly similar. Sequences in gut and soil of *A. gracilis* were significantly different from each other and from gut and soil of *G. paulistus*. However, gene analysis indicated a soil derived nitrate reducing gut microbiota for both earthworms, mainly consisting of members of the Rhizobiales. The collective results suggest that the emission of N_2O and N_2 is a common feature of earthworms. It remains unresolved whether gut size, feeding guild, or other factors contribute to the apparent inability of *G. paulistus* to emit nitrogenous gases.

SMV005

Anaerobic methane oxidizers prevent methane emissions from a minerotrophic peatland

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Freshwater sediments which receive nitrate fluxes from agricultural runoff and methane from methanogenesis theoretically provide ideal conditions for the recently discovered process of anaerobic methane oxidation coupled to denitrification. *Methylomirabilis oxyfera*, the responsible bacterium, employs a novel pathway, whereby N_2 and O_2 are formed from nitrite without N_2O as an intermediate; the oxygen is then used in the canonical aerobic methane oxidation pathway [1]. To further our understanding of the role of *M. oxyfera* in the environment, we determined methane and nitrate depth profiles in a minerotrophic peatbog during several seasons. Methane was depleted before reaching the oxic zone, and the depth where nitrate and methane coexisted displayed anaerobic methane oxidation activity. As measured by quantitiative PCR, also bacteria related to *M. oxyfera* were most abundant in this depth. It was subsequently used as an inoculum for an anaerobic, methanotrophic enrichment culture, using *in situ* water with nitrite and nitrate as electron acceptors and a pH of 6.2. During incubation, methane oxidation and nitrite conversion were regularly monitored. Stable-isotope experiments showed that nitrite was preferred over nitrate, and methane oxidation ceased without either electron acceptor. FISH microscopy and PCR amplification of the 16S rRNA (95% similarity) and particulate methane monooxygenase (pmoA) gene (90% similarity) revealed that new *Methylomirabilis*-like bacteria had been enriched. Taken together, these results suggest that novel *M. oxyfera*-like bacteria are responsible for methane depletion in the anaerobic zone of the investigated peatland.

[1] Ettwig et al. (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* **464**, 543-548.

SMV006

Microorganisms affecting the stabilisation of soil organic carbon in cryoturbated soils of the Siberian Arctic

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Permafrost underlies ~26% of terrestrial ecosystems and is estimated to contain around 50% of the world's soil organic carbon (SOC). A significant proportion of this SOC is stored in the subducted organic matter of cryosols. SOC decomposition in cryosols is strongly retarded suggesting that *cryoturbation* (= mixing of soil layers due to freezing and thawing) may be one of the most important mechanisms of Arctic carbon storage and long term stabilization. To eventually identify potential microbial key factors in the stabilization of SOC within cryoturbated soils, approximately a hundred soil samples were collected from three different landscapes in the East Siberian tundra (Cherskii, Northern Siberia; 69°N, 162°E). Samples covered organic topsoils, cryoturbated soils and its adjacent mineral horizons, and the underlying permafrost. Cryoturbated horizons showed similar soil characteristics as the topsoil horizons and were clearly distinguishable from the subsoils. Bacterial and archaeal abundances in cryoturbated horizons were found to be several orders of magnitude higher than in the surrounding mineral soils. However, the relative reduction of fungi in cryoturbations resulted in lower fungal:bacterial ratios compared to the top- and subsoil. This might be a key factor for elevated SOC stabilisation and its retarded decomposition in cryoturbated layers. Community profiling on the Illumina GAIIx genome analyzer identified members of the *Actinobacteria*, *Proteobacteria*, *Firmicutes* and the *Verrucomicrobia* as the most abundant phyla. Additionally, phylogenetic analyses revealed a community shift of potential indicator taxa and functional groups (e.g., *Firmicutes*, *Desulfuromonadales*) from the topsoil to the subsoil reflecting a change in redox conditions and a shift from aerobic/microaerophilic to anaerobic microorganisms. The community composition of cryoturbated soils was highly variable being rather similar to the subsoil or represent an intermediate stage from the top- to the subsoil. This variability presumably reflected differences in the parent soil, age and history of the cryoturbation and the degrees of SOC stabilisation.

SMV007

Could bacterial residues be an important source of SOM? - a case study from a glacier forefield

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Recently, stocks of soil organic matter (SOM) have been shown to decrease in European soils and also worldwide, which compromises soil fertility and enhances emissions of carbon dioxide and other, even worse green-house gases, to the atmosphere. However, the general structure of SOM, and thereby the mechanisms behind its genesis and loss, remain unclear.
In this framework, microbial biomass is generally regarded to be of low importance for SOM formation. In particular on freshly exposed surfaces, however, bacteria colonize barren mineral surfaces faster than fungi or higher plants. Moreover, recent results indicate that bacterial cell wall fragments frequently occur on soil mineral surfaces and also accompany the microbial colonization of previously clean and sterile activated carbon surfaces after incubation in groundwater. Hence, we hypothesized that, at least, in the initial stages of soil formation bacteria and their fragments may play an important role in particulate SOM formation bearing in mind that most dead organic matter entering the soil is processed by bacteria.
This hypothesis was proven by tracing the development of SOM in a chronosequence with samples from the forefield of a receding glacier (Damma-glacier, Canton Uri,Switzerland) by scanning electron microscopy and other methods. The initially barren mineral surfaces have been shown to be rapidly covered with microbial residues as soil age increases. Moreover, this data compares well to growing C/N-ratios, water contact angles and fatty acid contents in earlier deglaciated samples.

SMV008

Methanol Consumption by Methylotrophs in Temperate Aerated Soils

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Methanol is the second most abundant organic molecule in the atmosphere. The main source of atmospheric methanol is plant material. Methanol oxidation by aerobic microorganisms in soils might be an important sink in the global methanol cycle. Aerobic methylotrophs use methanol as a source of carbon and energy. Methanol oxidation kinetics were previously unknown. Currently, only few studies addressed structures of methanol-utilising microbial communities in aerated soils. Apparent Michaelis-Menten-Kinetics were experimentally determined in soil slurries that were supplemented with ^{14}C-methanol. $^{14}CO_2$ production was measured, and recovery of ^{14}C was calculated. Soil slurries with supplemental cyanide served as controls for abiotic activity, and were not substantially active compared to cyanide-free and methanol-supplemented slurries. Thus, methanol oxidation was primarily a biotic process. Washed roots from a grassland soil, and sterile grown Arabidopsis sp. plants exhibited lower methanol oxidation rates than root-free soil. Thus, not plant tissue but likely soil microorganisms were the main drivers of methanol oxidation. The $K_{M(app)}$ in a grassland soil (National Park Hainich) was 0.2 mmol per L. It is in the range of K_M-values of purified methanol dehydrogenases of the soil-borne methylotroph Hyphomicrobium denitrificans (0.2-168 mmol per L), which implies that likely methanol oxidation of the grassland soil was catalysed by methylotrophs. The in situ methylotroph community composition will be analysed in soil samples from the National Park Hainich by pyrosequencing of functional genes (mxaF, fae, mch) that are mandatory for methanol metabolism of methylotrophs. An analysis of mxaF genotype composition over the incubation period of the ^{14}C-methanol-experiment will provide information on responding key methylotrophs.

SMV017

Effects of elevated CO_2 concentrations on microbial ecosystem at the artificial test site ASGARD, England

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Increasing anthropogenic CO_2 emissions will lead to climate change and ocean acidification with severe consequences for ecosystems (Intergovernmental Panel on Climate Change, 2007). CO_2 capture and storage into geological formations like deep saline aquifers or depleted gas and oil reservoirs is one option to reduce greenhouse gas emissions.
As part of the EU funded "RISCS" project (Research into Impacts and Safety in CO_2 storage), a study investigating the impacts of potential CO_2 leakages on near-surface environments is being undertaken. To assess effects of potential CO_2 release at CO_2-non-adapted sites, microbial abundance, diversity and plant coverage at the ASGARD site (Artificial Soil Gassing and Response Detection, Nottingham) before, during and after CO_2 exposure are being studied.
Examination of environmentally important metabolic pathways and microbial groups showed clear differences between CO_2 injected plots with high (100%), medium (70%) and low (10%) CO_2 concentrations and control plots.
Increasing rates of methanogenesis and methane oxidation at high CO_2 concentrations were provided. CO_2 production rates as an important indicator for microbial activity showed decreasing trends under elevated CO_2 concentrations. Analysis of the microbial community composition by quantitative real time PCR and investigations of the microbial diversity (e.g. sequencing, TRFLP) illustrate alterations in microbial abundances under CO_2 influence.
Our results indicate a shift towards anaerobic and acid tolerant microbial populations.

SMV009

Evidence of aerobic polycyclic aromatic hydrocarbon (PAH) biodegradation in a contaminated aquifer by combining BACTRAP®s and laboratory microcosms.

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Polycyclic aromatic hydrocarbons (PAH) are among the most abundant groundwater contaminants, mostly as a result of petroleum and diesel spills and industrial processes. Due to their toxic, carcinogenic and mutagenic characteristics, cost-effective clean up strategies such as Monitored Natural Attenuation (MNA) are required for their removal from contaminated field sites. PAHs have been shown to be biodegradable despite the high activation energy needed to attack the aromatic ring and their tendency to sorb on hydrophobic surfaces thus hampering the biodegradation. Evidence for active PAH biodegradation *in situ* is difficult to obtain and requires suitable approaches for the routine application in the evaluation of NA potentials.
In this study, biodegradation of four polycyclic aromatic hydrocarbons (naphthalene, acenaphthene, fluorene, and phenanthrene) was demonstrated at a PAH-contaminated aquifer. *In situ* microcosms (BACTRAP®s) consisting of activated carbon pellets were loaded with [$^{13}C_6$]-naphthalene or [$^{13}C_5$/$^{13}C_6$]-fluorene (50:50) and incubated for over 2 months in monitoring wells to collect indigenous groundwater communities. Amino acids extracted from the developed microbial communities showed ^{13}C-incorporation of up to 30.4 atom%, thus demonstrating a highly active PAH-degrading microbial community at the field site. To further assess the biodegradation potential for the PAHs, laboratory microcosms were set up with [$^{13}C_6$]-naphthalene, [$^{13}C_5$/$^{13}C_6$]-fluorene (50:50), [$^{13}C_1$]-acenaphthene or [$^{13}C_1$]-phenanthrene. *In situ* microcosms exposed over a period of 99 days in field monitoring wells and groundwater samples served as inoculum for the laboratory microcosms. Analysis of ^{13}C-incorporation into the produced CO_2 using gas chromatography coupled to isotope ratio mass spectrometry (GC-IRMS) revealed a high degradation potential for all tested PAHs. The combined application of BACTRAP®s and laboratory microcosms can be a powerful tool for evaluating PAH biodegradation at subsurface impacted sites. The BACTRAP® system turned out to be suitable to study the degradation activity directly at the field site, but also facilitated enrichment of PAH-degrading communities for further laboratory cultivation experiments.

SMV010

Cobalt trace metal requirement for reductive dechlorination of trichloroethene by Dehalococcoides

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The genus *Dehalococcoides* plays a key role in the complete dechlorination of chlorinated ethenes because these bacteria are the only microorganisms known that are capable of reductive dechlorination beyond dichloroethene (DCE) to vinyl chloride (VC) and ethene. The reduction of chloroethenes by *Dehalococcoides* spp. is catalyzed by reductive dehalogenase (RDase) enzymes. The RDases in *Dehalococcoides* spp. are monomeric, vitamin B_{12}-dependent enzymes. A comparative genome analyses of trace element utilization in prokaryotes and eukaryotes revealed that *Dehalococcoides* have the largest cobalt-requiring metalloproteome among all sequenced prokaryotic genomes which is consistent with the high number of non-identical RDase homologs per genome (up to 36 in strain VS). Here we describe reductive dechlorination of trichloroethene (TCE) by a microbial mixed culture containing *Dehalococcoides* spp. in a defined mineral medium amended with varying concentrations of cobalt (0.6 μM to 2064 μM). We observed that elevated cobalt concentrations have a positive effect on cell growth and the rate of dechlorination by *Dehalococcoides* spp.. However, complete dechlorination of TCE to ethene and the highest cell yields were only obtained in enrichment cultures containing 36 μM cobalt. Enrichment cultures with significantly higher or lower cobalt concentrations showed mainly incomplete dechlorination leading to the accumulation of cis-DCE and VC. qPCR analysis showed that defined cobalt concentrations can lead to the selective enrichment of *Dehalococcoides* spp.. We also observed that *Dehalococcoides* containing different sets of chloroethene reductive dehalogenases react differently to cobalt. While 36 μM cobalt lead to the enrichment of VC and TCE reductive dehalogenase (*vcrA*/*tceA*)-containing *Dehalococcoides* other cobalt concentrations favoured only TCE reductive dehalogenase (*tceA*)-containing *Dehalococcoides* strains. Our experiments demonstrate how careful evaluation of findings from comparative genomics can further our understanding of the physiological requirements of environmental microorganisms with implications for their application in bioremediation.

SMV011

Anaerobic transformation of chlorobenzene and dichlorobenzene in highly contaminated groundwater

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The halogenated groundwater pollutants chlorobenzene (MCB) and dichlorobenzene (DCB) are ubiquitous in the environment and seem to be persistent and accumulating under anoxic aquifer conditions. However, our group could provide evidence for the transformation of chlorobenzene under anoxic conditions [1]. Futhermore Fung et al. [2]described the dehalogenation of DCB and MCB in anoxic microcosms.

Therefore, we hypothesize that both anoxic oxidation and reductive dechlorination may be parallelly occurring pathways for the removal of MCB and DCBin situ. This study aimed to investigate the microbial transformation of MCB and DCB in the complex environment of a constructed planted (*Juncus effusus*) model scale wetland. Additionally different redox conditions were compared in a laboratory microcosm study. After more than 365 days of continuous operation, the overall removal of MCB was >90% while DCB was completely removed in the model scale wetland. Concurrent sulphate and iron reduction was observed. The original groundwater pumped into the wetland was anoxic and contained ferrous iron and high concentrations of sulphate. Along the flow path, the geochemistry changed. We observed increasing sulphide and iron(II) concentrations in the anoxic and deeper sediment part whereas the upper zone became oxic and less sulfidic. In the microcosms, MCB mineralisation was observed under nitrate and iron reducing conditions. Microbial community analysis showed the presence of a diverse community which could be linked to methanogenic, sulphate or iron reducing (*Geobacter*) activity as well as to potential aerobic processes (*Burkholderia*). We identified representatives of the phylum *Chloroflexi* related to *Dehalogenimonas* which could be involved in the dehalogenation of chlorinated contaminants.

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2. Fung, J.M., et al.,Reductive dehalogenation of dichlorobenzenes and monochlorobenzene to benzene in microcosms.Environ Sci Technol, 2009.43(7): p. 2302-7.

SMV012

On the distinct physiological capabilities of so far uncultured archaea in acidophilic biofilms

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Biofilms can provide a number of different ecological niches for microorganisms. The here studied snotite biofilms in which pyrite oxidizing microbes are the primary producers are outstanding objects to study multispecies biofilms. This is due to their stability that allows in situ measurements as well as detailed fluorescence in situ hybridization (FISH) based characterization of the microbial population in different areas of the biofilm. Consequently, catalyzed reporter deposition (CARD) FISH was used to examine niches of archaea and bacteria in an acidic snotite biofilm. These results were combined with oxygen microsensor measurements to correlate the abundance of different phylogenetic groups to the available oxygen concentration. This concentration declined rapidly from the outside to the inside of the biofilm. Hence, part of the population lives under microoxic or anoxic conditions. *Leptospirillum ferrooxidans* strains dominate the microbial population but are only located in the oxic periphery of the snotite structure. *Acidithiobacillus* species were also detected but occurred in the oxic periphery as well as the anoxic core. Interestingly, archaea were identified only in anoxic areas of the biofilm. The archaeal community consists of so far uncultured *Thermoplasmatales* as well as novel ARMAN species. In addition to CARD FISH and oxygen microsensor measurements, in situ microautoradiographic (MAR) FISH was used to identify areas in which acitive CO_2 fixation took place. Leptospirilla as well as acidithiobacilli were identified as the primary producers. CO_2 fixation was revealed to proceed in the outer rim of the matrix. Hence, archaea inhabiting the snotite core do not seem to contribute to primary production. This work gives insight in the ecological niches of acidophilic microorganisms and their role in a consortium. The data suggests so far unprecedented capabilities of ARMAN species and can provide the basis for the isolation of so far uncultured archaea.

SMV013

Effects of sulfadiazine entering via manure into soil on abundance and transferability of antibiotic resistance in the rhizosphere of grass and maize

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Veterinary antibiotics introduced into soil via manure are assumed to promote the spreading of antibiotic resistance genes and selection of resistant bacterial populations. The rhizosphere is a hot spot of microbial interactions like horizontal gene transfer, as root exudates are a food source for microorganisms and a driving force of population density and activity. For example, it was shown that the addition of artificial root exudates increased the bacterial community tolerance towards the veterinary antibiotic compound sulfadiazine (SDZ) [1]. On the other hand, the exposure of bacteria to SDZ is presumably reduced in the rhizosphere since the dissipation of bioaccessible SDZ-concentrations was recently shown to be accelerated in rhizosphere soil, indicating an enhanced degradation of the compound [2]. However, so far little is known about the abundance and dynamics of sulfonamide resistance genes in the rhizosphere. We therefore compared the fate and effect of SDZ in bulk- and rhizosphere soil in mesocosms planted with maize and in field plots planted with maize or grass. In both experiments, manure was applied which was collected from pigs treated with SDZ or not. SDZ concentrations over time were analyzed by a sequential extraction protocol for soil yielding antibiotic fractions of different binding strength, which served as a proxy for the bioaccessible concentration. Following the application of manure, $CaCl_2$-extractable concentrations of SDZ and its metabolites tended to decrease faster in rhizosphere soil than in bulk soil whereas the dissipation rates of residual microwave-extractable SDZ were similar. Quantitative real-time PCR of total community DNA showed that the application of manure containing SDZ increased the relative abundance of the SDZ resistance genes *sul1* and *sul2* in bulk- and rhizosphere soil of maize, which may be associated with a propagation of LowGC-type plasmids. In the rhizosphere of the field experiment, the difference of relativesul abundance between the treatments increased over time, even at bioaccessible SDZ-concentrations below previously reported effective doses.

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2. Rosendahl, I.; Siemens, J.; Groeneweg, J.; Linzbach, E.; Laabs, V.; Herrmann, C.; Vereecken, H.; Amelung, W., Dissipation and Sequestration of the Veterinary Antibiotic Sulfadiazine and Its Metabolites under Field Conditions. Environmental Science & Technology 2011, 45, (12), 5216-5222.

SMV014

The 'rare biosphere' contributes to wetland sulfate reduction - fameless actors in carbon cycling and climate change

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Wetlands are a major source of the greenhouse gas methane and their response to global warming and increasing aerial sulfur pollution is one of the largest unknowns in the upcoming decades to centuries. Although regarded as primarily methanogenic environments, biogeochemical studies have revealed a hidden sulfur cycle in wetlands that can sustain rapid renewal of the small standing pools of sulfate. Here, we show by 16S rRNA gene stable isotope probing that a *Desulfosporosinus* species, which constitutes only 0.006% of the total microbial community, is a major sulfate reducer in a long-term experimental peatland field site when supplied with *in situ* concentrations of short-chained fatty acids and lactate. Parallel stable isotope probing using *dsrAB* [encoding subunit A and B of the dissimilatory (bi)sulfite reductase] identified no additional sulfate reducers under the conditions tested despite the high diversity of this functional marker gene in the studied peatland. Subsequent single substrate incubations revealed that sulfate reduction was stimulated best with lactate, propionate, and butyrate but not with acetate or formate. For the identified *Desulfosporosinus* species, a high cell-specific sulfate reduction rate of 341 fmol SO_4^{2-} $cell^{-1}$ day^{-1} was determined. Thus, the small *Desulfosporosinus* population has the potential to reduce sulfate *in situ* at a rate of up to 36.8 nmol (g soil w. wt.)$^{-1}$ day^{-1}, sufficient to account for a substantial part of sulfate reduction in the peat soil. Modeling of sulfate diffusion to such highly active cells identified no limitation in sulfate supply even at bulk concentrations as low as 10 μM. These data show that the identified *Desulfosporosinus* species, despite being a member of the 'rare biosphere', can contribute substantially to sulfate reduction, which diverts the carbon flow in peatlands from methane to CO_2 and, thus, alters their contribution to global warming.

SMV015

Microbial iron cycling in freshwater sediments

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Iron belongs to the dominant chemical elements in the Earth's crust and is therefore an important constituent in all environmental systems. Iron redox transformations and elemental cycling are strongly controlled by local geochemical conditions, as well as by the abundance and activity of iron-oxidizing and iron-reducing microorganisms. Applying a coupled geochemical-microbiological approach we attempted to determine the spatial distribution of the different iron transformation processes as a function of substrate, energy and electron donor/acceptor availability in freshwater sediments. As the microbial distribution is a function of local geochemical conditions we have determined the distribution of readily available electron acceptors (O_2, NO_3^-) and donors (Fe^{II}), as well as the abundance of iron-converting microorganisms with high spatial resolution. In addition, the bioavailable fractions of ferriferrous minerals were

determined as a function of the redox gradient. Moreover, the energy that is available to iron-converting microorganisms has been quantified with respect to local geochemical gradients. The combination of geochemical, mineralogical, energetical and microbiological data allowed a detailed investigation of the spatial structure of the iron cycling throughout natural redox gradients. First microcosm studies have been performed to investigate the competition for ferrous iron as electron donor for iron-oxidizing bacteria. The obtained data allow to construct a conceptual model describing the substrate and electron donor/acceptor flux between the areas of pronounced metabolic activity (i.e. different iron converting processes) in the elemental iron cycling throughout natural redox gradients and the interspecies substrate competition.

SMV016

Autotrophic Fe(II) oxidizing bacteria in the littoral sediment of Lake Große Fuchskuhle

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Lake Große Fuchskuhle is a dystrophic acidic bog lake located in northern Germany. The primary objective of this study was to investigate the processes and microorganisms responsible for anaerobic CO_2 fixation in the littoral sediment. A time-course DNA-SIP approach was used using $^{13}CO_2$. Little or no CH_4 production was observed during 12-week incubation, suggesting that conditions were not suitable for methanogenesis. Analysis of labeled 16S rRNA genes indicated that only a few species had incorporated the $^{13}CO_2$, including a group of Betaproteobacteria related to Gallionella and Sideroxydans species and a group within the Actinobacteria related to Acidimicrobium ferrooxidans. Previous studies have reported a high abundance of similar actinobacterial 16S rRNA sequences in this and other humic bog lakes, but the ecological function and physiology of these organisms is unknown. As most of the 16S rRNA genes sequenced from the heavy fraction are related to Gallionella, Sideroxydans and Acidimicrobium which are known iron oxidizing bacteria (FeOB), we investigated the possibility that the labeled organisms in this study were chemoautotrophic FeOB. Fe^{2+} concentrations were measured in the sediment and found to be 1.8 mM and enumeration by MPN method have shown the presence of $1x10^4$ autotrophic and $1x10^7$ heterotrophic FeOB in the sediment. Anaerobic enrichment incubations were performed and it was shown that the Actinobacteria could be highly enriched in the presence of Fe^{2+}, CO_2 and NO_3^-, suggesting they are autotrophic FeOB and could be using NO_3^- as a terminal electron acceptor. This study suggests that anaerobic chemoautotrophic FeOB may be dominant autotrophic bacteria in this lake and to our knowledge our results are the first to indicate the autotrophy and a probable Nitrate dependent ferrous iron oxidizing nature of theseActinobacteria.

SMP001

Microbial iron(II) oxidation in littoral freshwater lake sediments: Competition between phototrophic vs. nitrate-reducing iron(II)-oxidizers

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The temporal and spatial distribution of neutrophilic microbial iron oxidation is mainly determined by local physico-chemical gradients of oxygen, light, nitrate and ferrous iron. In the anoxic part of the top layer of littoral freshwater lake sediments, nitrate-reducing and phototrophic iron(II)-oxidizers compete for the same electron donor; reduced iron. Though a conceptual framework for biogeochemical iron cycling has been proposed[1], it is not yet understood how these microbes co-exist in the sediment, what their spatial distribution is relative to one another and what role they play in the overall iron cycle. In this study we show that both metabolic types of anaerobic Fe(II)-oxidizing microorganisms are present in the same sediment layer directly beneath the oxic-anoxic sediment interface. The photoferrotrophic MPNs counted $3.4 \cdot 10^5$ cells$\cdot g^{-1}$ and the autotrophic and mixotrophic nitrate-reducing Fe(II)-oxidizers totalled $1.8 \cdot 10^4$ and $4.5 \cdot 10^4$ cells$\cdot g^{-1}$ dry weight sediment, respectively. Additionally, in order to distinguish between the two microbial Fe(II) oxidation processes and to assess their individual contribution to the sedimentary iron cycle, littoral lake sediment was incubated in microcosms with various additives. We found that nitrate-reducing Fe(II)-oxidizing bacteria exhibited a higher maximum Fe(II) oxidation rate per cell in both pure cultures and microcosms than achieved by photoferrotrophs. However, where photoferrotrophs instantly started oxidizing Fe(II), nitrate-reducing Fe(II)-oxidizers showed a significant lag-phase in microcosms during which time they probably use organics as electron donor before they initiated Fe(II) oxidation. This suggests that nitrate-reducing Fe(II)-oxidizers will be outcompeted by photoferrotrophic Fe(II)-oxidizers during optimal light conditions due to Fe(II) limitations, as phototrophs deplete Fe(II) before nitrate-reducing Fe(II)-oxidizers start Fe(II) oxidation. Thus, the co-existence of the two anaerobic Fe(II)-oxidizers may be possible due to a niche space separation in time by the day night cycle, where nitrate-reducing Fe(II)-oxidizers oxidize Fe(II) during the night and phototrophs play a dominant role in Fe(II) oxidation during daylight hours. Furthermore, metabolic flexibility of Fe(II)-oxidizing microorganisms may play a paramount role in the conservation of the sedimentary Fe cycle.

1. C. Schmidt, S. Behrens and A. Kappler, Environmental Chemistry7(2010), p399-405

SMP002

Intermediary Ecosystem Metabolism in Different CH_4-emitting Peatland Soils

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Natural wetlands such as bogs and fens contribute up to approximately 40% to the global emission of methane. Biopolymers in peatland soils are anaerobically degraded via intermediary events that terminate in the emission of methane (i.e., collectively 'intermediary ecosystem metabolism'). Glucose, acetate, and H_2-CO_2 have been observed to stimulate intermediary events (i.e., fermentation, acetogenesis) and terminal events (i.e., methanogenesis) in anoxic microcosms of soils from the regional fen Schlöppnerbrunnen. The stimulation of glucose-, acetate-, and H_2-CO_2-dependent processes were analyzed in different regional peatland soils and compared to the intermediary ecosystem metabolism of the fen Schlöppnerbrunnen. Peatland soils were diluted with mineral medium and incubated in the dark under anoxic conditions. The microbial community in soil microcosms were evaluated with *mcrA*/*mrtA* (encode for the alpha-subunit of methyl-CoM reductases I and II) and bacterial 16S rRNA genes. Glucose-dependent fermentation was stimulated in all soil microcosms, but product profiles differed between sampling sides. Propionate, butyrate, and CO_2 accumulated as end products in all soil microcosms. Ethanol, H_2, and acetate accumulated as end products in soil microcosms from some peatland soils or were partially degraded in others. Formate was transiently detected in glucose-supplemented soil microcosms from some peatland soils. Hydrogenotrophic methanogenesis was stimulated in all soil microcosms, whereas acetoclastic methanogenesis and H_2-dependent acetogenesis were stimulated in most of the soil microcosms. Most abundant taxa under *in situ* conditions in one of the fen soils were *Acidobacteria*, *Anaerolineae*, unclassified and novel taxa, whereas *Acidobacteria*, *Alphaproteobacteria*, unclassified and novel taxa were most abundant in one of the peat bog soils under *in situ* conditions. Methanogens of the contrasting soils were also resolved. The collective results reinforce the likelihood that the intermediary ecosystem metabolism differs between different peatland soils and that *Acidobacteria*-related taxa as well as hitherto unknown taxa are integrated to the 'intermediary ecosystem metabolism' and the emission of methane in the peatland soils.

SMP003

Mobilization of cadmium from Fe(III) (oxyhydr)oxides during microbial Fe(III) reduction in cadmium-contaminated soil

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Soils worldwide have increasingly been contaminated with industrial waste metals, including cadmium, which may subsequently enter the food chain through agriculturally used plants. These contaminant metals influence the natural ecosystem drastically and can have dramatic effects on human health. Hence, there is a need for the development and application of new techniques to efficiently remediate contaminated soils. In the study presented here, we combined phytoremediation and microbially enhanced natural attenuation to determine whether a more time- and cost-efficient removal of cadmium from contaminated sites is achieved. A cadmium-tolerant Fe(III)-reducing bacterium of theGeobactergroup was enriched and isolated from a highly cadmium-contaminated site in Germany. By designing specific primers the isolatedGeobacterstrain was quantified in cadmium-contaminated sites and laboratory experiments. In batch experiments this cadmium-tolerant Fe(III)-reducer was shown to mobilize cadmium from Fe(III) (hydr)oxides through reductive dissolution. Subsequently, the phytoavailable cadmium was actively be taken up by the metallophyte cadmium hyperaccumulator plantArabidopsis halleriand accumulated in the above ground tissue. In plant-microbe-soil mesocosms, geochemical and microbial parameters were determined to trace the microbial release of cadmium from cadmium-bearing Fe(III) minerals by the natural microbial community in comparison to sterile setups. Additionally, the cadmium uptake and accumulation by the plantA.halleriin the presence and absence of these bacteria was quantified. By harvesting the plant regularly, an efficient removal of cadmium from contaminated sites may be achieved.

SMP004

Physiological constraints of microbial electron shuttling from bacteria via redox-active humic substances to poorly soluble Fe(III) minerals

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Microbial redox processes in soils and sediments impact biogeochemical cycling of elements and nutrients and are controlled by the availability of different electron acceptors. Reduction of Fe(III) poses a challenge to microbes, since Fe(III) is present at neutral pH in form of poorly soluble minerals. However, Fe(III)-reducing bacteria are known to overcome this solubility problem and several mechanisms have been suggested for electron transfer from the outer membrane to the surface of the ferric (oxyhydr)oxides: (i) direct electron transfer from outer membrane c-type cytochromes requiring direct cell-mineral contact, (ii) indirect reduction of Fe(III) via solubilization of the Fe(III) by organic chelators and uptake and reduction of the Fe(III) in the cell, or (iii) indirect reduction of the Fe(III) minerals via electron shuttles such as dissolved or solid-phase humic substances (HS) (Konhauser et al., 2011). HS are ubiquitous in the environment and can be used by a variety of microbes as electron acceptor as well as electron mediator to transfer electrons from the cell to other electron acceptors. However, it is currently unknown whether both mineral-surface-associated and planktonic cells benefit from HS as electron mediators and how microbes, minerals and HS are spatially arranged as a function of cell density.
We studied the extent of microbial reduction of the Fe(III) mineral ferrihydrite [$Fe(OH)_3$] by *Shewanella oneidensis* strain MR-1 at different concentrations of cells in the presence and absence of HS. As expected, HS stimulated Fe(III) reduction in high-cell-number systems in which excess planktonic cells transfer electrons via dissolved HS to the Fe(III) mineral surface that is otherwise inaccessible to them. Unexpectedly, we found that the presence of HS also stimulated Fe(III) reduction in low-cell-number systems where all cells present had direct access to the mineral surface. This suggests that even small spatial gaps between electron-releasing cell-surface proteins and the mineral surface can be bridged via redox-active HS. Confocal laser scanning microscopy (CLSM) was used to image cell-mineral-HS-aggregates and to visualize how the microbial cells were distributed in the setups. These results emphasize the relevance of HS in biogeochemical redox processes in soils and sediments.
Konhauser, K.O., Kappler, A., Roden, E.E. (2011) Iron in microbial metabolism. Elements, 7, 89-93.

SMP005

Studying colonization on stone surfaces by a model biofilm in a flow-through chamber approach

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Soil formation on weathering rock surfaces is intrinsically connected with primary microbial colonization at the atmosphere-lithosphere interface. Rock-inhabiting life is ubiquitous on rock surfaces all around the world, but the laws of its establishment, and more important, quantification of its geological input are possible only in well-controlled and simplified laboratory models. In a previous study [1] a model rock biofilm consisting of the heterotrophic black yeast *Sarcinomyces petricola* and the phototrophic and nitrogen-fixing cyanobacterium *Nostoc punctiforme* was established.
In the present work the growth of this model biofilm on diverse materials with different physical and chemical properties was investigated under well controlled laboratory conditions. To clarify the role of environmental factors, the parameters temperature, light intensity, CO_2 content and relative humidity were varied in growth test series. For an accelerated stone colonization and to increase the biomass yield different flow-through chamber systems with semi-continuous cultures have been applied, simulating weathering conditions like flooding, desiccation and nutrient input. The biofilm development was studied by (i) light and electron microscopy and (ii) qualitatively and quantitatively with respect to cell forms and biomass. Mixed and single cultures of the model biofilm protagonists were compared to elucidate possible growth influencing effects by the respective symbiotic partner.
Under the mentioned environmental conditions two types of flow-through chambers have been applied (i) with a neutral growth supporting membrane and (ii) including mineral materials to explore possible rock surface colonization. The first flow-through chamber type is directly observable under the light microscope and can be divided into two compartments via a semi-permeable membrane allowing co-cultivation of single cultures and a regular control of their cell morphology. With this system it is possible to determine if a metabolite exchange between the model biofilm partners is sufficient for the symbiosis or if there is a need for a direct cell-cell-contact. Possible biologically induced mineral surface alterations were followed on various rock substrates exposed in the second flow-through chamber system.
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SMP006

Composition of methanogenic archaea of the El'gygytgyn Crater Lake NE-Siberia

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Arctic lakes are an important source of methane, which has a 26 times stronger greenhouse gas effect than CO_2. An increase of abundance and surface of North Siberian lakes has lead to a rise of methane emission up to 58% from 1974 to 2000 [1]. Nevertheless, the knowledge about methane dynamics in arctic lakes and methanogenic archaea in deeper sediment deposits is still limited.
To deepen the understanding of methane dynamics in arctic lakes, a molecular biological characterization of methanogenic archaea was carried out in 10.000 to 400.000 years old sediment deposits of the El'gygytgyn Crater Lake drilled in scope of the ICDP-project 'Scientific Drilling in El'gygytgyn Crater Lake' [2]. Archaeal DNA was successfully amplified throughout deposits of Middle and Late Pleistocene as well as Holocene. Furthermore, on the base of 16S rRNA, denaturing gradient gel electrophoresis and clone libraries of selected samples showed a diversity of methanogens affiliated with *Methanosarcinales*, *Methanocellales* and *Methanomicrobiales*. The methanogenic diversity strongly varied throughout the sediment depths with two areas of high diversity in 250.000 and 320.000 years old sediments. Additionally, a positive correlation between the diversity and the amount of organic carbon was discovered. Application of propidium monoazide helped to distinguish between viable cells and free DNA and showed that a great proportion of amplified DNA came from intact cells. The oldest living archaea was isolated out of 390.000 years old sediment deposits. Moreover, a higher methane production rate was detected in areas of high diversity. Conclusively, methanogenic archaea are able to survive in a metabolic active stage over hundreds of thousand years under lake conditions. As the temperature of deeper lake sediments is rising in the context of climate change, it is to be expected that their activity and consequently the methane emission will increase. Summarising the results give valuable insights in the methane dynamic in deeper sediment deposits and confirm the influence of old carbon source to positive feedback loop of climate change.
[1] K.M. Walter et al., Nature **443** (2006), p. 71-75.
[2] M. Melles et al., Scientific Drilling **11** (2011), p. 29-40.

SMP007

Population analysis and Fluorescence in situ Hybridisation of aerobic chloroethene degrading bacteria

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Chloroethenes are a major source of groundwater and soil contamination. Several mixed cultures and pure bacterial strains, which grow under anaerobic conditions using chloroethenes as electron acceptor in addition to auxiliary substrates, have been published and examined in regard to application as bioremediation agent. However, aerobic microorganisms that use the target pollutant like vinyl chloride (VC) as growth substrate would be favourable for bioremediation processes.
This study has the aim to identify microorganisms involved in aerobic degradation of chloroethenes to use them for bioremediation of contaminated sites.
Different species were identified via 16S-rRNA PCR-DGGE experiments using eubacterial primers and subsequent sequence analysis using BLAST of the NCBI database. Based on these results species specific primers for PCR and specific gene probes for fluorescence in-situ hybridisation (FISH) were designed.
According to the sequence analysis results, five different species, which belong to the ß-proteobacteria sub-family, were identified in metabolic chloroethene degrading batch cultures inoculated with ground water samples of contaminated sites. Focussing on these species, conventional PCR approaches with specific primers were performed.
FISH analysis can determine the presence of the specific bacteria in samples from the contaminated site using the previously designed gene probes. For that, a preceding treatment regarding the cell wall permeability was established and the hybridization conditions were optimized for each FISH probe. To verify the quality and specificity of the gene probes different reference bacteria were used as positive and negative control.
Quantitative PCR experiments targeting possible candidate genes for chloroethene biotransformation will be performed for molecular characterization of aerobic decontamination processes.

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SMP008

Will be presented as SMV017!

SMP009

Role of nitrite accumulation and mineral nucleation sites for Fe(II) oxidation by the nitrate-reducing *Acidovorax* sp. strain BoFeN1

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Anaerobic, neutrophilic nitrate-reducing Fe(II)-oxidizing bacteria can be found in anoxic environments and were suggested to play a key role in iron mineral formation and N-cycling under these conditions. In order to understand the coupling of the microbial iron and nitrogen cycles in anoxic environments as well as the effect of mineral nucleation sites on iron mineral formation, we conducted batch experiments with the nitrate-reducing, iron(II)-oxidizing bacterium *Acidovorax* sp. BoFeN1, which was isolated from anoxic littoral sediments in Lake Constanze [1].
During denitrifaction nitrite can be formed, which is known to oxidize Fe(II) abiotically [2]. This raises the question whether the oxidation of ferrous iron is indeed enzymatically catalyzed or whether it is just a chemical reaction as a consequence of microbial nitrite formation during acetate oxidation by the mixotrophic Fe(II)-oxidizing strains. In order to shed light on this question we grew strain BoFeN1in the absence and presence of iron and quantified nitrite formation during denitrification. Additionally, we incubated BoFeN1 with nitrous oxide (N_2O) as electron acceptor and either acetate or acetate/Fe(II) as electron donors to circumvent the problem of nitrite formation. These experiments showed that microbially formed nitrite contributes significantly to Fe(II) oxidation and has to be considered in the overall Fe(II) oxidation budget.
In order to identify the influence of mineral surfaces of microbial Fe(II) oxidation products, we used ^{57}Fe-specific Mössbauer spectroscopy and ^{57}Fe(II)-spiked growth medium in combination with seeding minerals of natural isotopic composition to identify the mineral products formed from the dissolved Fe(II) during Fe(II) oxidation. Analysis of Mössbauer spectra of microbial products showed that in the absence of nucleation site minerals, strain BoFeN1 produces goethite (α-FeOOH). The presence of magnetite (Fe_3O_4) induced the formation of magnetite besides goethite while the presence of hematite (α-Fe_2O_3) nucleation sites did not induce hematite formation but only goethite was formed. This study showed that mineral formation not only depends on geochemical conditions but can also be controlled by the presence of mineral nucleation sites that initiate precipitation of certain mineral phases.

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SMP010

Molecular characterization of nitrogen-fixing bacteria and their colonization pattern in mangrove roots

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Nitrogen-fixing bacteria play a major role in re-mineralization processes in mangrove ecosystems. Anaerobic processes like denitrification take place in the anoxic layers of mangrove sediments. Consequently, most of the nitrogen is lost and thus no longer available for metabolic processes in plants. Previous studies had shown that nitrogen-fixing bacteria interact with mangrove roots making nitrogen available for plants. Although, nitrogen fixation is a very important process in mangrove ecosystems, very little is known about bacterial colonization strategies and physiological impacts on mangrove roots. Additionally, virtually nothing is known about bacterial genes particularly required and expressed during the interaction of bacteria with mangrove plants. The establishment of a nitrogen-fixing bacterium-mangrove interaction model system is necessary to study the molecular mechanisms of this interaction. The aim of the current investigation was to first isolate and characterize nitrogen-fixing bacteria associated with root material of *Avicennia sp*. and *Rhizophora mangle*. Subsequently, the colonization patterns of selected bacterial strains on mangrove roots had to be investigated. Nitrogen-free medium was used for the isolation of 9 bacterial strains assigned to two different phylogenetic classes. Isolates were characterized in terms of their ability to fix atmospheric nitrogen, their phylogenetic affiliation using 16S rRNA gene sequencing, their genetic accessibility, and their ability to survive and colonize mangrove roots when inoculated with different other sediment-borne indigenous bacterial strains (fitness test). The mangrove root colonization patterns of two isolates, *Halomonas sp*. and *Vibrio sp*., were followed by confocal laser scanning microscopy. Herein, it was demonstrated that some of the diazotrophs were genetically accessible and were colonizing mangrove plants. These isolates are promising candidates to establish a cell-to-cell bacteria-mangrove model system to continue our investigation of the molecular mechanisms determining bacteria-mangrove interactions.

SMP011

Effect of Oxygen Availability on Microbial Chitin Degraders in an Agricultural Soil

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Chitin is a biopolymer consisting of alternating β-1,4-linked N-acetylglucosamine residues, and is the second most abundant organic compound of terrestrial biomass. In an unsaturated soil, oxygen distribution is highly heterogeneous, and dynamic on the micro- to millimeter scale. Therefore, different redox processes, such as fermentation or oxygen respiration, can simultaneously be active on the degradation of chitin. In a wheat-planted soil from Klostergut Scheyern,oxygen availability impacted differentially on the activation of redox processes and activity of bacterial taxa during cellulose degradation. The objective of the current study was to evaluate the effect of oxygen availability on microbial processes and taxa during degradation of chitin and its hydrolysis products N-acetylglucosamine and glucosamine. Supplemental chitin, N-acetylglucosamine, and glucosamine were completely mineralized to carbon dioxide under oxic conditions. Concentrations of ammonium and nitrate increased in the chitin-supplemented treatment, which suggested a release of ammonium by ammonification, and subsequent oxidation of ammonium to nitrate by nitrifiers. Chitin, N-acetylglucosamine, and glucosamine were anaerobically metabolized to carbon dioxide, molecular hydrogen, methane, acetate, propionate, and butyrate. Nitrate was completely consumed during the experiment, and the soil microcosms went black, which indicated precipitation of ferrous iron. Thus, respiration of nitrate and ferric iron by soil microbes was active. The findings suggest that oxygen availability differentially activated redox guilds (aerobes, fermenters, nitrate and ferric iron reducers) during the degradation of chitin. The identity of the activated chitinolytic taxa is currently under investigation by analysis of 16S rRNA and chitinase genes.

SMP012

Ammonification and nitrification rates depend on soil and land use type of subtropical savannah soils

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Drylands (i.e., arid, semiarid, and subhumid areas) cover approximately 40% of earth´s terrestrial surface and the percentage increases due to climate change. However, over one billion people depend on agriculture in these disadvantaged regions. Besides water supply, nutrients like ammonium and nitrate limit plant production in these areas. In the present study, ammonification and nitrification - N-liberating processes that are mainly driven by microorganisms - were quantified by the Pool Dilution Technique (PDT). In this approach, ^{15}N-ammonium and ^{15}N-nitrate are added to the soil to increase the $^{15/14}$N ratio and changes of the $^{15/14}$N ratio during an incubation experiment allow the calculation of gross ammonification and nitrification rates. Sampling sites were located in North-Eastern Namibia south of the Okavango river. The soil samples differed with respect to soil type (sand, i.e., Kalahari sands, and loamy sand, i.e., old flood plain soils) and land use type (fallow, drought and irrigation agriculture, bushland, and riparian woodland). First results of the PDT indicate that microorganisms responsible for ammonification and nitrification seem to be mainly influenced by soil type rather than land use type. Ammonification rates were highest in woodlands on loamy sands and lowest in fallow and drought agricultures. In contrast, nearly no ammonification and nitrification was detected in the Kalahari sands. These results are in agreement with CO_2 production rates which were highest in woodland soils from old flood plains, indicating highest microbial activities in these undisturbed soils.

SMP013

Culturability of novel *Acidobacteria* in German grass- and woodland soils

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Acidobacteria on average account for 20% of all soil bacteria and are physiologically active *in situ*. Culture-independent studies indicate that *Acidobacteria* are nearly as diverse as the *Proteobacteria* and currently comprise 26 distinct phylogenetic subdivisions (sd). However, until now only a few strains from sd 1, 3, and 8, have been validly described. Genome analysis revealed the ability of *Acidobacteria* to use complex

substrates as carbon sources. Within the German Biodiversity Exploratories project we focus on functional interrelations between *Acidobacteria* and land use. Six extensively managed sites from the Exploratories Schwäbische Alb, Hainich-Dün, and Schorfheide-Chorin, one grassland and one woodland soil per exploratory, were selected for a high throughput cultivation approach. Microtiter plates were inoculated with 10 and 50 cells per well, respectively, using five media atin situpH. The media tested contained (i) highly diluted carbon sources (HD1:10), (ii) low amounts of sugars, fatty acids, and amino acids (C-Mix), (iii) soluble humic acids (e.g., sodium salicylate, furfural, phthalic acid), (iv) insoluble humic acids (e.g., quercetin, coumestrol, solanine), and (v) a mix of polymeric substrates (e.g., chitin, pectin, cellulose). Culturability of total aerobes ranged from below 0.2% (Schorfheide-Chorin, grassland, soluble humic acids) to 9.2% (Schwäbische Alb, woodland, HD1:10). *Acidobacteria*-positve wells were identified via a specific PCR approach. The percentages of cultured *Acidobacteria* among all cultured *Bacteria* ranged from 0% (Schorfheide-Chorin, woodland, polymeric substrates) to 19.5% (Schwäbische Alb, woodland, polymeric substrates). The *Acidobacteria* recovered were affiliated with sd 1, 3, 4, and 6. In polymers-supplemented media, only representatives of sd 1 were detected. In contrast, most members of sd 6 *Acidobacteria* were cultivated in C-mix medium. For both, total aerobes and *Acidobacteria*, cultivation success was highest with media containing easily available carbon sources, indicating that low amounts of these substrates favor growth of soil bacteria, in particular *Acidobacteria*.Characterization of novel *Acidobacteria* as relevant members of the soil microbial community will improve our knowledge about biogeochemical cycling in soils.

SMP014
Carbon Isotope Fractionation of Italian Rice Field Soil under H_2/CO_2 and different temperature regimes.

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In anoxic environments organic matter is fermented to short chain fatty acids, alcohols as well as CO_2 and H_2. The two gaseous products can be further converted to either methane by methanogenic archaea or to acetate by acetogenic bacteria. Methanogenesis is energetically more favourable than acetogenesis. Nevertheless acetogens can outcompete methanogens at low temperatures. To investigate the contribution of both processes we incubated anoxic rice slurry under H_2/CO_2 at 15°, 30° and 50° C and followed the isotopic signatures of the carbon compounds (CO_2, CH_4, acetate) by mass spectrometry. For better differentiation of the two processes a second incubation was performed with bromoethanesulfonate an inhibitor of methanogenesis.

SMP015
Methanogens at the top of the world

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Deserts (semiarid, arid and hyperarid regions) cover around one third of the Earth´s surface. Desert soils are typically covered by a unique layer termed biological soil crust (BSC), a few millimetres thick and densely colonized by microorganisms. During dry periods the BSC is mostly inactive, but following wetting the microbial activity increases and oxygen becomes limiting. It was previously shown that BSC from hot deserts can then produce methane (1). We wanted to investigate whether this phenomenon can also be observed in high-altitude cold deserts in the Himalayas (Ladakh, India). For this purpose, soil samples from three different vegetation zones: semiarid, steppe, and subglacial, as well as from front and lateral moraines of a receding glacier were collected and tested for the production of methane.
We incubated 5 g soil with 5 ml water at 25 °C under anoxic conditions and followed up gas production (CH_4, CO_2 and H_2) and the isotopic signature of the carbon in the CH_4 and CO_2. Almost each sample from the vegetation zones produced methane, and also some from the moraine transects. Methane production was faster in the BSC compared to the deeper soil layers, demonstrating that most methanogens are likely to be concentrated at the top layer. The isotopic analysis showed that methane probably developed from both acetate and CO_2 with no significant difference between the layers. Our results demonstrate the existence of an active methanogenic community even at such extreme oxic environment.

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SMP016
Community analyses of fermentative hydrogen producers in environmental samples

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Fermenters produce Hydrogen (H_2) to excrete excess reductant. Fermentative H_2 production is catalyzed by either [FeFe]-hydrogenases (e.g., during butyrate fermentation of *Clostridium butyricum*) or Group 4 [NiFe]-hydrogenases (e.g., during mixed acid fermentation of *Escherichia coli*). Similarity correlations between in silico translated amino acid sequences from publicly available hydrogenase genes and corresponding 16S rRNA genes showed that closely related hydrogenases (i.e., ≥ 80% amino acid sequence similarity) belonged to host organisms within the same family. However, due to gene duplication and subsequent diversification, distantly related hydrogenases did not necessarily belong to hosts of different families. Degenerate primers targeting [FeFe]- and Group 4 [NiFe]-hydrogenase genes were developed to identify potentially active hydrogen producers in environmental samples. [FeFe]-hydrogenase gene sequences obtained from a methane emitting fen were affiliated to the *Clostridia*, *Alpha-* and *Deltaproteobacteria*, *Chloroflexi*, *Bacteroidetes*, *Verrucomicrobia* and *Negativicutes*. Group 4 [NiFe]-hydrogenase gene sequences obtained from H_2-emmiting earthworm gut content were affiliated to the *Gammaproteobacteria*, *Clostridia* and *Verrucomicrobia*. These results demonstrated that the new hydrogenase primers are useful for the detection of a wide range of [FeFe]- and Group 4 [NiFe]-hydrogenases in environmental samples and that 80% amino acid sequence similarity is a reasonable cut-off to group hydrogenases from fermentative hydrogen producers on the family level.

SMP017
Electrochemical Quantification of Microbial Humic Substance Reduction

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Humic substances (HS) are ubiquitous in soils, sediments and waters and have been shown to shuttle electrons between microorganisms and poorly soluble electron acceptors such as Fe(III) minerals. Since HS can be reduced by a variety of microorganisms including Fe(III)-reducing, sulfate-reducing and dechlorinating bacteria, but also chemically for example by sulfide, electron transfer via HS has the potential to contribute significantly to the electron fluxes in the environment. While microbial HS reduction has been studied for a variety of different HS and microorganisms, these results were semi-quantitative due to indirect quantification of HS redox states.
We quantitatively followed the microbial reduction of HS of different origin (soil, peat, and aquatic) by the dissimilatory Fe(III)-reducing bacterium *Shewanella oneidensis* MR-1 using mediated electrochemical reduction and oxidation. Microbial HS reduction resulted in a decrease in the number of electrons that could be transferred to the HS electrochemically (electron accepting capacity, EAC) and in a concomitant increase in the number of electrons donated from the same HS to the working electrode (electron donating capacity, EDC). Thus, microbial HS reduction could be shown and the amount of electrons transferred from the microbes to the HS could be quantified over time. Aeration of the cultures with air resulted in an increase in the EAC and a decrease in the EDC, indicating the re-oxidation of the previously reduced moieties in the HS. Subsequently, the HS were re-reduced by the bacteria as could be seen in a decrease in the EAC and increase in the EDC. These findings demonstrate the reversibility of the microbial HS reduction. Throughout the entire experiment, the sum of EAC and EDC remained constant, demonstrating that microbial reduction did not alter the total number of redox active moieties in the HS. Overall, our results provide important new quantitative insights into the extent of microbial HS reduction and give new indications about the significance of this process in environmental systems: HS redox reactions can contribute significantly to the (trans)formation of iron minerals and the (im)mobilization and reductive degradation of organic and inorganic pollutants and to the redox buffer capacity of systems such as peats.

SMP018
Biogeography of soil *Burkholderia* populations

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The genus *Burkholderia* is an important component of soil microbial communities and comprises over 60 species. *Burkholderia* species have a

broad distribution in nature, occurring commonly in soil and in association with plants, fungi and animals, where mutualistic as well as parasitic interactions can be found. The importance of *Burkholderia* species as opportunistic pathogens (e.g. in cystic fibrosis patients) is increasingly recognized and the molecular mechanisms underlying virulence have been extensively studied. However, little is known so far about the abundance, the diversity and the biogeography of the genus *Burkholderia* in natural environments such as soils. Reports from the literature indicate that *Burkholderia* species are often isolated from acidic environments, which suggests that pH could be an important factor in shaping the biogeography of *Burkholderia*. To assess this question, 46 soil DNA samples collected across North and South America were used (Fierer and Jackson, 2006). A specific real time PCR protocol targeting *Burkholderia* 16S rRNA gene was developed to analyse the relative abundance of *Burkholderia* sp. in these soil samples. Results suggest that pH has a significant effect on *Burkholderia* relative abundance in soils: the highest relative abundance was observed in soils ranging between pH 5 and pH 6 where up to 6.7 % of total bacterial 16S rRNA genes were represented by *Burkholderia* species. Lower pH soils also showed high relative abundance of *Burkholderia* (up to 2.8%). However, *Burkholderia* 16S rRNA copy numbers were not detected in alkaline soils. We are currently investigating the diversity of *Burkholderia* in a subset of 15 selected sites varying in pH, C:N ratio, location of sampling and relative abundance of *Burkholderia*. This will allow us to better understand which populations are particularly affected by pH and which other factors are shaping the abundance, the diversity and the biogeography of soil *Burkholderia* species.

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SMP019

Characterization of microbial dehalogenation using compound specific stable isotope analysis

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Chlorinated ethenes are the most common soil and groundwater contaminants worldwide. Technical application of tetra- and trichloroethenes (PCE, TCE) in the dry cleaning industry and metal degreasing resulted in big scale production and release in the environment. Chloroethenes are an issue of serious risk for human health and suspected to be carcinogenic. During the last decade several bacterial strains were isolated from contaminated soil and groundwater capable of reductive dehalogenation of chlorinated ethenes. However, the actual reaction mechanism and the involved genes responsible for specific dehalogenation are still a point of interest.

We aim to investigate and characterize the reductive dehalogenation reaction in several strains, using stable isotope techniques. Compound specific stable isotope analysis can be used to analyze the reaction mechanism and degradation pathway. Previously, we observed that the carbon stable isotope fraction was highly variable when comparing different strains capable of the reductive dehalogenation of the chlorinated ethenes [1]. These differences may either be due to differences in enzyme mechanism or to e.g. rate limiting effects and substrate uptake processes as observed for *Sulfurospirillum multivorans* and *Desulfitobacteriumsp*. strain PCE-S [2]. Our preliminary experiment have shown that rate limitation does not appear to play a role in *Dehalobacter restrictus* and that highly similar enzymes, although present in different organisms i.e. *D. restrictus* and *Desulfitobacterium* sp. PCE-S, produced similar isotope effects, contrary to previous publications. Further studies should allow analysis of the causes for different isotope fractionation of the chlorinated ethenes by other bacteria such as *Geobacter* or *Desulfuromonas*.

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SMP020

Abundance, distribution, and activity of Fe(II)-oxidizing and Fe(III)-reducing microorganisms in hypersaline sediments of Lake Kasin, Southern Russia

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The extreme osmotic conditions prevailing in hypersaline environments result in decreasing metabolic diversity with increasing salinity. Various microbial metabolisms have been shown to occur even at high salinity, including photosynthesis, sulfate and nitrate reduction. However, information about anaerobic microbial iron metabolism in hypersaline environments is scarce. We studied the phylogenetic diversity, distribution, and metabolic activity of iron(II)-oxidizing and iron(III)-reducing bacteria and archaea in iron-rich salt lake sediments (Lake Kasin, Southern Russia; salinity 348.6 g L^{-1}) using a combination of culture-dependent and -independent techniques. 16S rRNA gene clone libraries for Bacteria and Archaea revealed a microbial community composition typical for hypersaline sediments. Most probable number experiments and enrichment cultures confirmed the presence of microbial iron(II) oxidation and iron(III) reduction in the salt lake sediments. Microbial iron(III) reduction was detected in the presence of 5 M NaCl, thereby extending the natural habitat boundaries for this important microbial respiratory process. Quantitative real-time PCR showed that 16S rRNA gene copy numbers of total Bacteria, total Archaea, and species dominating the iron(III)-reducing enrichment cultures (relatives of Halobaculum gomorrense, Desulfosporosinus lacus, and members of the Bacilli) were highest in an iron oxide-rich sediment layer. Combined with the presented geochemical and mineralogical data, our findings suggest the presence of an active microbial iron cycle at salt concentrations close to the solubility limit of NaCl.

SMP021

Microbial, geochemical, and mineralogical contributions to arsenic removal from drinking water in house hold sand filters in Vietnam

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Worldwide more than 100 million people ingest detrimental concentrations of arsenic by consuming groundwater contaminated from natural geogenic sources. Many Asian countries, in particular Vietnam, Bangladesh, India, and Cambodia are known to be affected by high groundwater arsenic concentrations as a result of chemically reducing aquifer conditions. Household sand filters are simple to operate and remove on average 80% of arsenic from groundwater containing 1 mg/L of ferrous iron or an iron/arsenic ratio of about 50. The installation and operation costs of household sand filters are low and the construction materials are locally available. The filters can treat a reasonable amount of groundwater within a short time and they can easily be installed by the affected communities. Oxidation of dissolved iron present in the groundwater leads to the formation of sparsely soluble iron(hydr)oxide particles in the sand filters, which bind negatively charged arsenic species and reduce arsenic concentrations in the water. Although household sand filters have been proven to be an effective technical solution for mitigating arsenic exposure, not much is known about microbial iron, manganese, arsenic redox-processes occurring in the filters and their effect on filter efficiency. Therefore, one of the goals of this study was to isolate, identify, and quantify Fe, Mn, and As-oxidizing and -reducing microorganisms from a arsenic removal sand filter and to study their specific Fe, Mn, and As redox activities. Water samples and filter solids were collected from a local sand filter close to the city of Hanoi, Vietnam. The samples were geochemically and mineralogically characterized. Total iron, arsenic, manganese, and phosphate concentrations, pH, TOC, TIC measurements, as well as total cell counts were performed on samples from various depth of the sand filter. Most probable number counts confirmed the presence and activity of various iron, manganese, arsenic redox-processes and their distribution within the water filter. The goals of this research project are to better understand the microbial redox transformation processes that drive arsenic/manganese/iron mineral interactions in household sand filters and to give recommendations for improved filter use and filter material disposal.

SMP022

Impact of Biochar Amendment on Microbial Nitrogen-Cycling in Agricultural Soil

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N_2O is a major greenhouse gas (GHG) contributing 8% to global GHG emissions with agricultural sources representing 84% of anthropogenic N_2O emissions. N_2O is a product of microbial denitrification and its formation is correlated to fertilizer use. Soil biochar amendment has been observed to decrease soil N_2O emissions. Biochar is a stable, carbon rich product that is manufactured by thermal decomposition of organic material under limited oxygen supply. Although the effect of biochar on nitrous oxide emissions from soil has been studied previously the mechanisms behind the reduced N_2O emission from biochar amended soil are not yet understood. We investigated whether the decrease in N_2O emissions caused by biochar amendment is due to changes in the functional composition of the nitrogen-cycling microbial community. For this reason agricultural soil was incubated in microcosms with different amounts of biochar under oxic (60% WFPS - water filled pore space) and anoxic (100% WFPS) conditions over a period of 3 month. Copy numbers of different functional genes involved in the microbial nitrogen cycle were quantified by real-time PCR. Gene abundance and expression were correlated to N_2O, CO_2, CH_4 emissions as well as soil NH_4^+, NO_2^- and NO_3^- concentrations. Differences in microbial respiration rates in the presence of various nitrogen compounds in the treatments with and without biochar were quantified in BIOLOG assays. Our experiments

showed reduced N_2O emissions in the biochar treatments up to 84%. In general N_2O emissions were 30 times higher under anoxic conditions compared to the emissions from the oxic microcosms. Decreased N_2O emissions were correlated to an increase in the relative abundance of nitrous oxide reductase (nosZ) gene copy numbers during the first two weeks after biochar addition. Our results further showed that reduced N_2O emissions from biochar amended soils were directly linked to changes in the functional composition, nitrogen compound utilization, and activity of the nitrogen-transforming microbial community. Overall, soil biochar amendment promoted complete denitrification via stimulation of growth and activity of nosZ-containing, nitrous oxide reducing denitrifiers. Our findings will facilitate the development of new mitigation strategies for anthropogenic GHGs.

SMP023

Microbial and metabolic analysis and optimization of CH_4 production from wheat stillage at elevated temperatures

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Development of an efficient bioethanol production plant based on biomass requires the integration of various biological and non-biological processes. After the bioconversion of wheat to ethanol and the distillation process high amounts of lignocellulose and dead yeast cells still remain untreated (stillage). From a high-temperature biogas plant, using corn and chicken manure as biogas substrates, a microbial community was enriched able to convert wheat stillage to methane at 55°C.
In order to investigate the microbial community in the model biogas reactor pyrosequence analysis of 16S rRNA gene-tags was conducted resulting in 23,000 gene sequences. The studies indicate the predominance of Archaea of the genera *Methanosaeta* and *Methanothermobacter* and Bacteria of the families *Thermotogaceae*, *Anaerolineaceae*, *Synergistaceae* and *Thermodesulfobiaceae*.
The archaeon *Methanothermobacter thermautotrophicus* and the bacteria *Lutispora thermophila, Thermoanaerobacter thermosaccharolyticum, Clostridium succinogenes* and *Thermohydrogenium kirishiense* were isolated by anaerobic serial dilution techniques
To improve biogas production we investigated different strains for their bioaugmentation potential. We could demonstrate that the addition of a pure culture of *Caldicellulosiruptor saccharolyticus* to the existing biogas community resulted in a considerable biogas intensification rate with a constant CO_2/CH_4 ratio.
To investigate dynamics and stability of the microbial community during process modifications such as increase in loading rate and addition of accumulating intermediates, denaturing gradient gel electrophoresis and fluorescence in-situ hybridization was employed. The results revealed the presence of a robust consortium of methanogenic Archaea.
The set of primers developed in this study provides a tool for monitoring methanogenic communities from a wide range of biogas processes.

SMP024

Methanogenic communities and their response to Holocene and Late Pleistocene climate changes in permafrost environments

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The currently observed climate change due to global warming is expected to have a strong impact, notably on Arctic permafrost environments. The thawing of permafrost is suggested to be associated with a massive release of greenhouse gases, in particular methane. Thus, Arctic permafrost regions play a fundamental role within the global carbon cycle and the future development of Earth's climate. To understand how the system will respond to climate changes it is not only important to investigate the current status of carbon turnover but also how the system reacted to climate changes in the past.
This presentation therefore takes a journey through time from the recent active layer of permafrost to Holocene and Late Pleistocene permafrost deposits in the Siberian Arctic, to reconstruct the microbial driven methane dynamics. Generally, in-situ methane contents of the deposits reflect the TOC profile with depth underlining the correlation of the distribution of organic matter and methanogenesis. Significant amounts of methane could also be found in Late Pleistocene deposits of an age of 30 and 41 ka, respectively. Lipid biomarkers and amplifiable DNA were successfully recovered throughout the whole permafrost sequences with an age of up to 42 ka. Analysis of the abundance and distribution of archaeol, an indicator for fossil methanogenic communities, revealed a temperature response to climate changes during the Late Pleistocene and Holocene. Past warming trends seem to cause an enhancing of methanogenic communities, while cooling trends conversely caused them to decrease. Furthermore, indications for recently living archaeal communities in frozen ground could be found, using phospholipid ether lipids (PLEL) and 16S rRNA fingerprints as specific markers. The obtained data on present and past methanogenic communities suggest a response to future warming events as was reconstructed from previous warmer periods.

SMP025

FISH in soil: applications for the *in situ* investigation of microorganisms

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Fluorescence in situ hybridization (FISH) represents a powerful method for the phylogenetic identification, enumeration, and visualization of single microbial cells in soil. The applicability of this tool for studies in soil microbiology is exemplarily shown on the basis of several FISH approaches which are used in our lab.
For routine applications of FISH in soil, the amplification of fluorescent signals (CARD) is necessary for a clear discrimination of target signals from the intense background induced by organic matter, high contents of clay, and plant tissue. CARD-FISH in soil provides quantitative data of single microbial cells, and thus gives insight into the composition of microbial consortia associated with different microenvironments (e.g. bulk soil and rhizosphere).
With CARD-FISH applied to roots, the enumeration of single cells as well as the analysis of the spatial distribution of these microbes on the rhizoplane gives additional information for comprehensive studies in the soil-root interface. Especially on roots, sequential hybridizations with fluorochromes of different spectral characteristics have shown to be useful for the analysis of microorganisms on domain specific or hierarchic levels.
The combination of FISH and micropedology (resin embedding and thin sectioning of soil samples) allows for the *in situ* detection of single microorganisms in the undisturbed soil matrix. The simultaneous use of multiple oligonucleotide probes thereby provides information on the spatial distribution of microorganisms belonging to different taxonomic divisions.
In the recently developed gold-FISH protocol, conjugates labelled with fluorochromes and nanogold particles allow the combinative approach of analyzing microorganisms by epifluorescence and scanning electron microscopy. It is therefore possible to identify and localize single microbial cells *in situ* on an ultrastructural level. Furthermore, the biochemical conditions in the microbial habitat of gold-FISH detected cells can be characterized by element mapping generated with energy dispersive X-ray spectroscopy.

SMP026

Bacterial communities change along a glacier forefield transect - A combined approach of molecular fingerprints (T-RFLP) and environmental analyses

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Glacier forefields are known to be a pioneer site for primary succession and inhabit extreme climatic and environmental conditions. Retreating glaciers expose new terrestrial terrain that becomes accessible for soil formation and microbial colonisation. Pioneer microorganisms support weathering processes and the colonisation of more complex microbial communities or plants. Because increasing temperatures due to climate change enhance glacial degradation, it is important to understand how bacterial communities react to changing environmental conditions. A combined approach of geochemical and microbiological examinations will be used to describe the habitat characteristics and the complex system of microbial communities in two glacier forefields on Larsemann Hills, East Antarctica. Terminal Restriction Length Polymorphism Analyses show that bacterial community structures vary significantly between glacier forefields of different development status. Although relatively low, enzyme activities increase with an advanced forefield development and decrease with increasing depth. The extreme habitat conditions become apparent within the geochemical and geochemical properties. The study site is characterised by very low nutrient and water availability and a coarse grain size. Statistics reveal a connection between environmental and biological data and the position of the sample in the glacier forefield. Altogether our results show a high abundance and variability of microorganisms in the hardly developed habitat glacier forefield.

SMP027

Contrasting assimilators of 2,4-dichlorophenol derived carbon occur in soil and drilosphere

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Chlorophenols are frequently detected in terrestrial and freshwater ecosystems, are carcinogenic, mutagenic, and recalcitrant. Thus, chlorophenols are of major environmental concern. 2,4-dichlorophenol (2,4-DCP) has been extensively used as wood preservative, is an intermediate in the degradation of the herbicide 2,4-dichlorophenoxyacetic acid, and was chosen as model compound. Degradation occurs in soil, mainly due to aerobic microbial processes. Degradation 'hot spots' in soils include the drilosphere, i.e., earthworm gut content, cast, and burrows. Assimilators of [$U^{13}C$]-2,4-DCP were identified in soil columns with and without soil-feeding earthworms (Aporrectodea caliginosa). [^{12}C]-2,4-DCP treatments served as controls for SIP. Disappearance of low, in situ relevant concentrations of 2,4-DCP (20 μg g_{DW}^{-1}) was stimulated by earthworms. Barcoded amplicon pyrosequencing coupled to 16S rRNA stable isotope probing (SIP) yielded app. 337,000 sequences and identified 39 family level taxa of 2,4-DCP-[^{13}C] assimilators relative to [^{12}C]-2,4-DCP controls. Alpha-, Beta-, Gamma - and Deltaproteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, Chloroflexi, Actinobacteria and Cyanobacteria were 2,4-DCP-[^{13}C] labelled. Sphingomonadaceae, Comamonadaceae, Pseudomonadaceae and Flavobacteriaceae dominated assimilators of [$U^{13}C$]-2,4-DCP. Many detected 16S rRNA genes were only distantly related to publicly available sequences. Labelled Clostridiaceae were exclusively detected in the anoxic earthworm gut and cast, labelled Flavobacteriaceae occurred only in cast, labelled Pseudomonadaceae dominated in burrow walls, while labelled Sphingomonadaceae were detected in earthworm-unaffected soil only. [$U^{13}C$]-2,4-DCP 16S rRNA SIP of enrichment cultures set up with soil and drilosphere material from soil coulmn experiments indicated that Comamonadaceae and Sphingomonadaceae reacted to continuous supply over a long time period of 2,4-DCP (50 days, pulsing of 5 x 60 μg g_{DW}^{-1} every 10 days) in liquid media. The collective data indicates that (a) earthworms stimulate the degradation of 2,4-DCP in soil and drilosphere, (b) earthworms select for distinct microbial degraders of 2,4-DCP in the drilosphere, and (c) soils influenced by earthworms harbor highly diverse and hitherto unknown 2,4-DCP-utilizing microorganisms. Thus, vermiremediation may prove to be an environmentally sustainable way to treat contaminated soils.

SMP028

Behaviour of actinomycete communities in soils fertilized with biotransformed Dry Olive Residue

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The Spanish olive oil industry generates huge amounts of Dry Olive Residue (DOR). This waste is a major environmental problem. Despite its potential fertilizer value, its incorporation into soil results in a variety of negative effects related with its toxicity caused by several phenolic substances (Sampedro *et al*., 2004). These and others components have been linked with the phytotoxic and antimicrobial this residue's effects (Paredes *et al*., 1986; Linares *et al*., 2003; Sampedro *et al*., 2008). Treating this waste with saprobic fungi could transform DOR into organic fertilizer (Sampedro *et al*., 2005). Knowledge about the impact of biotransformed DOR on soil microbial ecology is scarce. This work aimed to characterize the physico-chemical properties of DOR bioremediated with the saprobic fungus *Coriolopsis rigida* after 30 days of incubation at 28ºC and to measure the effect of the application of this residue for 0, 30 and 60 days on the actinomycete communities of sorghum rhizospheric soil. The total polyphenols in *C. rigida*-treated DOR decreased by 75% and the C/N was reduced. The concentration of some microelements like aluminium, iron, chrome and zinc was significantly differed. To further understand the effects of residues on the actinomycete communities, 200 bacterial strains were isolated in gellan gum-solidified VL70 modified medium (Sait *et al*., 2002; Joseph *et al*., 2003; David *et al*., 2005) from a control soil, a soil incubated with untreated DOR and soil incubated with *C. rigida*-treated DOR over time. The population of filamentous actinomycetes from each treatment were morphologically distinguished. Soil populations of actinomycetes were 90% lower in soil amended with untreated DOR respect to the control soil across all times. However, this decrease was less evident in the soil treated with *C. rigida*-treated DOR. The 16S rRNA gene of all the actinomycetes was analyzed with actinomycete-specific primer (Xiao *et al*., 2011) by PCR-DGGE. The data showed that *C. rigida*-treated DOR applied to soil reduced the negative impact on actinomycete microbial communities.

SMP029

Characterization of the microbial community in a deep saline aquifer used for geothermal heat storage - Thermal effects on microbial composition and microbial induced corrosive and precipitative processes affecting plant operation

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The microbial diversity of a saline aquifer (mineralisation 131 g/L) used for geothermal heat storage in the North German Basin was characterized over a period of two years. Results of SSCP- and DGGE- fingerprinting and scanning electron microscopic (SEM) analyses indicated distinct differences in the microbial community composition in the cold and the warm well, with temperatures ranging between 45-54°C and 65-80°C, respectively. High temperature in the warm well probably enhanced organic matter availability and set off a cascade of organic matter transformation favouring diverse heterotrophic bacteria in the warm well and fermentative bacteria after temperature reduction due to heat extraction in the topside facility. In contrast, a high diversity of sulphate reducing bacteria (SRB), affiliated to members of the genera Desulfotomaculum, Desulfohalobium and Candidatus Desulforudis audaxviator, was detected in the cold well. They were accounted for the corrosion damage to the submersible pump in the cold well and iron sulphide precipitations in the near well bore area and topside facility filters of the cold well. This study reflects the thermal effects on microbial composition in a geothermally used aquifer, whereas the microbial induced processes adversely affect plant operation; and this applies particularly to the cold well.

SMP030

Influence of manure and Sulfadiazine on microbial diversity pattern and the distribution of resistance genes against antimicrobials in an artificial rhizosphere

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Sulfadiazine (SDZ) is a veterinary antibiotic which is widely used in animal livestock. Farming animals excrete residues of SDZ and resistant microbiota which are introduced into the environment by manure application to arable land. This process is argued to lead to the spreading of antibiotic resistance genes in the environment and finally causes increasing untreatable bacterial infections in humans. The plant root surface - also called rhizoplane - is a hotspot of microorganisms due to plant root exudation. Bacterial cells attached to the plant root form community-like structures enclosed by an extrapolymeric matrix. Therefore, microorganisms are protected from abiotic and biotic stresses and due to the high proximity of the cells the chance for horizontal gene transfer increases. This leads to our hypothesis, that the rhizoplane could be an ideal environment for spreading antibiotic resistance genes.
To address this question, we used an artificial root model (ARM) which consisted of a glass slide covered with an artificial root exudate mix incubated in soil. For pre-incubation, both piggery manure and SDZ were added to the soil, and after one and two weeks incubation of the ARM respectively, we harvested the attached microorganisms. To detect the influence of manure and SDZ application to the bacterial communities on the ARM, we performed T-RFLP based on 16S rRNA genes and quantified the SDZ-resistance genes *sul1* and *sul2* by quantitative PCR. The results show an increase of SDZ resistance in bacterial communities attached to the ARM, but no shift in community structure when preincubated with manure and SDZ.

SMP031

Metabolic networks in soil microbial communities investigated by protein-stable isotope probing (protein-SIP)

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The degradation of plant-derived materials like leaf litter, consisting of cellulose, lignin, hemicellulose, pectin and proteins, is an interesting subject to study complex mineralization cycles in nature [1]. Consortia of bacteria and especially fungi greatly contribute to this key ecosystem process by expressing a suite of various extracellular enzymes. In fact, these microorganisms decompose almost 90% of the plant biomass

produced in terrestrial ecosystems [2]. The goal of the present project is to provide a closer insight towards the structure and function of these communities by identifying metabolically active species, interactions and metabolic networks. For the detection of metabolic key players protein-SIP is applied, a method based on the metabolic incorporation of isotopically labeled substrates, e.g. with ^{13}C, ^{15}N or ^{36}S, into the proteome of microorganisms [3].
Protein-SIP experiments were performed in which soil from a tobacco field in Germany was incubated with leaf litter from either ^{15}N-labeled tobacco or ^{13}C-labeled corn plants as substrate over 14 days. The microbial growth within the approaches was monitored by measuring the biological oxygen demand. Immediate oxygen consumption was measured in the leaf litter-soil incubations and sampling took place three times in the first three days and three times within the remaining 11 days. The samples were conducted to two protein extraction steps: one for the extracellular and another one for the intracellular proteome. Proteins were separated by 1-dimensional SDS gel electrophoresis and peptides were analyzed by UPLC Orbitrap MS/MS measurements. For protein identification the metagenome sequence of the soil from the tobacco field was conducted. 454 pyrosequencing resulted in about 390 Mb distributed over about 871,000 reads with an average length of 450 bp. MG-RAST analysis showed that a large proportion of the functional genes belong to bacterial proteins (~97%) and to eukaryotic proteins (~2%). In addition to the assessment of the phylogeny of organism in the soil the metagenome will facilitate the identification rate of the metaproteome approach and therefore will increase the number of proteins for which the ^{13}C and ^{15}N incorporation patterns can be determined.

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SMP032

Horizontal gene transfer in wastewater irrigated soils in the Mézquital Valley, Mexico

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The Mézquital Valley (60 km north of Mexico City) is the world´s largest wastewater (WW) irrigation area. There, untreated WW from Mexico City is reused for crop irrigation. This practise might pose risks for field workers and consumers of agricultural products, because of the presence of pharmaceuticals, pathogens and antibiotic resistance genes in the WW. We performed soil column experiments with two different types of soil (soil irrigated with WW for 100 years and rain-fed soil) to investigate the spread of resistance genes by horizontal gene transfer (HGT) in WW irrigated soils. To visualize plasmid transfer an Enterococcus faecalis donor harbouring a mobilizable broad host range plasmid labeled with the Green Fluorescent Protein (GFP) [1] and a second non-mobilizable plasmid labelled with the Red Fluorescent Protein (RFP) [2] were added to rain-fed and 100 years-irrigated soil, each in soil columns of 20 cm height and 15 cm diameter. The mobilizable plasmid contains a replication origin for Gram-positive and Gram-negative bacteria, the gfp gene under control of the inducible nisin promoter and the pIP501 origin of transfer. Soil columns were irrigated once a week, in total three times. At each irrigation 10^9 donors were applied to the columns with one pore volume of artificial rainwater (for rain-fed soil) or WW (for WW-irrigated soil). During irrigation leachate water was collected. Furthermore pore water was sampled at 4 heights with suction cups. Soil samples from the top were taken daily. After 4 weeks soil samples were taken from different heights (every 2.5 cm). Bacteria in soil and water which have acquired the mobilizable resistance plasmid via plasmid transfer are detectable through their green fluorescence while their donors are identified by their green and red fluorescence. Transfer rates for both types of soil and in water will be presented. The soil column experiment will help assess the risk posed by HGT of resistance determinants in WW-irrigated soil.

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SMP033

Understanding factors which shape the community of nitrifiers: structural and functional analyses

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Understanding factors which drive the ecology of microbial communities involved in nitrogen turnover is of central importance for sustainable land use. As a model system grassland sites treated with different land use intensities were studied: (I) intensely used meadows, (II) intensely used mown pastures and (III) extensively used pastures. Samples were taken in spring and in the summer to investigate the seasonal as well as the land use intensity effect. In the last years it was found that in many soils ammonia-oxidizing archaea (AOA) are more abundant than ammonia-oxidizing bacteria. However, till now the contribution of AOA to total ammonia turnover rates are not clear. In order to address this question we estimated a theoretical potential nitrification rate (PNR) based on the actual measured abundances of archaeal and bacterial ammonia monooxygenase genes (amoAAOA andamoAAOB) and hypothetical maximum oxidation rate constants. This approach offers the possibility to estimate not only the theoretical PNR values but also the respective contributions of AOA and AOB. A comparison between the theoretical and the measured PNR values shows that they fit quiet well together. In order to assess the correlation between the observed temporal changes in nitrification activities, but also the found variability between the single grassland plots, a diversity analysis based onamoAAOA genes was performed. The results showed that the single treatments are statistically well separated but surprisingly no clear differences between the two time points could be found. Summarizing, our results strike out that AOAs deliver a high ammonium turnover potential to the soils. Changes in nitrification potentials are seemingly not due to AOA diversity, but driven by the activity state of the AOAs, which probably has changed between spring and summer. Based on the above results, we assume that diversity of theamoAAOA gene is shaped by long-term changes in environmental parameters, whereas the activity is probably driven by seasonal changes of environmental conditions.

SMP034

Metagenomic and metatranscriptomic analysis of German soil samples

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Phylogenetic, transcriptomic, and functional analyses of microbial communities present in soil samples from the German Biodiversity Exploratories Schorfheide-Chorin, Hainich-Dün, and Schwäbische Alb were performed (see www.biodiversity-exploratories.de). The experimental procedure included the isolation of whole genomic DNA from the A horizon and B horizon of selected forest and grassland sites. The prokaryotic diversity present in the different samples was assessed by pyrosequencing of amplicons using hypervariable regions of 16S rRNA genes as target. Differences in prokaryotic community compositions between A- and B-horizons as well as between forest and grassland samples were detected. Additionally, we extracted total RNA from soil samples, enriched mRNA, and used it for the synthesis of cDNA. Pyrosequencing of the generated cDNA and subsequent sequence analysis allowed to assess soil microbial gene expression profiles.
Metagenomic small-insert and large-insert libraries were constructed using genomic DNA extracted from the different soil samples. Comparative screening of the libraries for key microbial functions, such as cellulolytic, hemicellulolytic, and lipolytic activities was carried out. Several clones expressing cellulase-, hemicellulase-, and lipase/esterase- activity were obtained during function-driven screening of the libraries. Genes encoding (hemi)cellulolytic or lipolytic activity were recovered from the corresponding clones and sequenced. So far, analyzed (hemi)cellulolytic enzymes were assigned to glycosidhydrolase families 9 and 11. Thirty-five of the 37 analyzed lipases/esterases grouped into superfamilies I, IV, V, VI, and VIII of lipolytic enzymes. The remaining two represent putatively novel families. Biochemical characterization of (hemi)cellulolytic and lipolytic enzymes was carried out.

SMP035

Characterization of *Paenibaciilus polymyxa* RCP6 isolated from root nodules of Blue pea

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Question: *Clitoria purpurea* L. (blue pea) is a slender climber legume known for its beautiful bluish-crimson coloured flowers. This is found

growing all over the North, Central and East India in a variety of habitats and soil types. The capability of native bacterial strains from root nodules to behave as plant growth promoting bacteria and biocontrol agents was investigated.

Methodology: Isolation of root-nodule symbiont (Palnaippan *et al*., 2010)
Basic characters of isolate (Palnaippan *et al*., 2010)
Genomic DNA isolation, 16S rRNA amplification, 16S rRNA sequencing, Phylogeny and accession number (Palnaippan *et al*., 2010)
Plant Growth Promoting Characters (Li *et al*., 2008)
Antagonistic characters (Senthilkumar *et al*., 2009)
Germination Assay

Results: One strain (RCP6) over 21 isolates from the root nodules of *C. purpurea* were able to grow on Ashby`s N free media over seven successive generation indicative of presumptive N_2-fixation, an IAA producer, solubilised organic P from calcium phytate, able to release water soluble inorganic phosphate from tri-calcium phosphate, di-calcium phosphate and zinc phosphate with organic acid production on MM9 medium, show halo on $ZnPo_4$ tris-minimal media indicative of zinc solubilisation apart from zone on Aleksandrov`s medium exhibiting solubilisation of potassium. Isolate had the capability to antagonize *Macrophomina phaseolina*, *Fusarium udum*, *F. oxysporum*, *F. solani*, *Rhizoctania solani*, *Sclerotinia sclerotiorum* in dual culture as well as cell-free culture filtrate but show no activity against *Colletotrichum* spp. Conventional identification tests, Hi-media Carbokit™ indicated that RCP6 behaves like the *Paenibacillus* genus. Molecular identification by 16S rRNA sequence analysis identified the strain as *Paenibacillus polymyxa*. The 1492 base pair sequence of *P. polymyxa* RCP6 (GU369972) showed maximum similarity to *P. polymyxa* IAM 13419T (D16276). Strain also showed the ability to improve early vegetative growth of *C. purpurea* in germination assay.

Conclusion: This study disclosed features of Plant growth promoting and antagonistic strain *P.polymyxa* RCP6 that deserve further studies aimed at confirming its importance as a putative endophyte.

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SMP036

Competition between subalpine plants and microbes for nitrogen under different redox conditions and nitrogen fertilization regimes - a greenhouse approach

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Natural grasslands are important hotspots for biodiversity and other ecosystem services of soils. The gramineous species of these natural grasslands differ greatly in nitrogen uptake strategies: Whereas exploitative plants need high amounts of nitrogen compounds for growing, conservative plants require lower amounts. So far, the influence of plant nitrogen uptake strategies on microbial community is largely unknown. However, it can be hypothesized that the microbial rhizosphere community of exploitative plants differ from that of conservative plants due to high competition between exploitative plants and microbes for available nitrogen.

The aim of this study was to investigate the microbial rhizosphere community of subalpine gramineous plants with different nitrogen uptake strategies. Furthermore, the influence of low oxygen content due to high soil water content was examined, as anoxic conditions are known to favour denitrification processes and thus might facilitate microbes during the competition for nitrate. Therefore, a greenhouse experiment with Achillea millefolium(exploitative), Bromus erectus (intermediary) and Briza media(conservative) was performed in sandy, nutrient poor soil. Plants received 40 kg NH_4NO_3 ha^{-1} after 7 days and 60 kg NH_4NO_3 ha^{-1} after 21 days of growth. After 28 days plants were sampled. The microbial rhizosphere community was investigated by quantification of functional genes involved in nitrification (bacterial and archaeal amoA) and denitrification (nirK,nirS and nosZ) by real-time PCR. Soil ammonium and nitrate concentrations were determined. Furthermore potential enzyme activities of nitrification and denitrification were analyzed. The presentation will give detailed results on the allocation pattern.

SMP037

Population analysis of iron depositing bacterial communities in technical water systems

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This subproject of the BMBF project „Antiocker“ focuses on the identification and characterization of iron depositing bacteria under neutral pH. Iron bacteria have caused problems in water since the 19th century and there have been many references to red water becoming undrinkable presumably due to the growths of iron bacteria. The aim of this project is to identify the key bacteria which are involved in deposition of oxidized iron compounds. Their activity becomes a very important economic concern as a result of the intense deposition of iron oxides in technical water systems. Examples are the processing of groundwater, drinking water production or operation of water wells. Therefore ochreous samples from several technical water systems were examined to get an overview of the composition of the bacterial population.

For this purpose, traditional cultivation techniques such as bacterial isolation and molecular methods like PCR-DGGE, FISH in combination with epifluorescence-and confocal laser scanning microscopy were combined. The isolation of different iron precipitating bacteria has been successful and their living conditions can be characterized now. In addition to that a 16S rDNA genomic clone library of seven different samples from ochreous water wells (opencast mine and well reactors) was generated. 384 clones based on the 16S rDNA are available to make a molecular evolutionary analysis. Besides classical iron bacteria like *Gallionella* and *Leptothrix*, representatives of typical soil bacteria of the genera *Sphingomonas*, *Novosphingobium*, *Hyphomicrobium* and *Arthrobacter* were inside.

Based on this data, different specific oligonucleotide probes and primers will be developed for iron bacteria to detect them in their natural habitat and make a fast sample screening possible.

SMP038

Hydrolytic bacteria involved in degradation of plant biomass in the biogas process

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As fossil energy supplies are on a decline, technologies that employ regrowing resources have become of mutual interest. Biogas plants employ such resources as substrate for microbial fermentation processes which converte the contained energy in the energy carrier biogas. Improvement of these processes is of general interest. The aim of this work is to get insights into the composition of hydrolytic bacteria in biogas plants to optimize the hydrolysis of lignocellulosic material. This leads to improved methan yield and increased efficiency of the biogas process. We focused on the development of hydrolytic mixed cultures, their analysis and the development of monitoring methods to investigate the abundance of hydrolytic bacteria in (inoculated) biogas fermenter. Another approach was to purify cellulolytic cultures to investigate their capabilities.

SMP039

Selective transport of bacterial populations through the vadose zone during groundwater recharge

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Plants introduce abundant carbon into soil, were it can be sequestered in microbial biomass and recalcitrant organic matter. However, proportions of these pools can be relocated, by event-driven transport to deeper vadose zones and even to the groundwater during groundwater recharge, such as heavy rainfalls or after snowmelt. It is postulated that large fractions of this efflux are biocolloids, or microbial biomass in specific. Relevant questions are, whether only selected microbial populations are exported from top soils and what is the fate of this biomass in deeper zones and groundwater. Is it merely a carbon input for subsurface microbial food webs or do transported populations survive?

Here, at an agricultural experimental field site, we analyzed the composition of mobile bacterial communities collected in seepage water directly after recharge events at different depths (35 and 65 cm) and compared it to the corresponding bacterial communities from soil and vadose depths. Using T-RFLP and high-throughput pyrotag sequencing, we reveal that top soil bacteria are washed out selectively, and that their fate in deeper zones may be distinct, but taxon-specific. These findings greatly extend our understanding of the event-driven and organismic flow of carbon from soil into the subsurface.

SMP040

amoA-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of *amoA* genes from soils of four different geographic regions

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Ammonia-oxidizing archaea (AOA) play an important role in nitrification and many studies exploit their *amoA* genes as marker for their diversity and abundance. We present an archaeal *amoA* consensus phylogeny based on all publicly available sequences (status June 2010) and provide evidence for the diversification of AOA into four previously recognized clusters and one newly identified major cluster. These clusters, for which we suggest a new nomenclature, harbored 83 AOA species-level OTUs (using an inferred species threshold of 85% *amoA* identity). 454 pyrosequencing of *amoA* amplicons from 16 soils sampled in Austria, Costa Rica, Greenland, and Namibia revealed that only 2% of retrieved sequences had no database representative on the species-level and represented 30-37 additional species-level OTUs. With the exception of an acidic soil from which mostly *amoA* amplicons of the *Nitrosotalea* cluster were retrieved, all soils were dominated by *amoA* amplicons from the *Nitrososphaera* cluster (also called group I.1b), indicating that the previously reported AOA from the *Nitrosopumilus* cluster (also called group I.1a) are absent or represent minor populations in soils. AOA richness estimates on the species level ranged from 8-83 co-existing AOAs per soil. Presence/absence of *amoA* OTUs (97% identity level) correlated with geographic location, indicating that besides contemporary environmental conditions also dispersal limitation across different continents and/or historical environmental conditions might influence AOA biogeography in soils.

SMP041

Organic propagation of olive nursery plants using *Pantoea eucrina* strain AG9

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The demand for organic olive oil is increasing exponentially each year (MARM, 2010). However, nowadays there is not a commercial method that can replace the use of a synthetic hormone in organic propagation of olive nursery plants.
The goal of this work is the development of an organic olive propagation method based on the use of the strain AG9 (Pantoea eucrina), previously characterized as a Plant Growth Promoting Rhizobacteria (PGPR). This strain has been used in monoxenic model systems showing both, higher rooting induction and root elongation than negative controls, in mung bean and canola assays. Moreover, this bacterium has been tested in four olive cultivars, under nursery conditions, displaying higher or similar effectiveness than the hormonal treatments. These results underwrite the worth of this method.
On the other hand, by means of Confocal Laser Scanning Microscope (CLSM) and GFP tagged bacterial cells, it was confirmed that this strain is able to colonize the plant roots permanently as an endophyte and to promote the plant growth a long-term.
MARM. Anuario de Estadística. Ministerio de Medio Ambiente, Medio Rural y Marino. Gorbierno de Espana (2010).

SMP042

Temperature effects of geothermal energy use on the microbial community in subsurface environments

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Geothermal energy use has boomed in the last years. In winter, the exploitation of geothermal energy can be used to heat buildings and in summer to cool them. Especially geothermal heat exchangers up to 100 m depth are installed frequently. Although geothermal energy use is accepted as being environmentally friendly, several aspects need to be considered in an evaluation of its use. While complications during drilling (e.g. leading to cracks in houses) or during the operation of geothermal heat systems (e.g. leakage of anti-freezing agents to the aquifer) are caused by misjudgment or accidents, other effects cannot be avoided.
Generally, temperature in the subsurface deeper than 15 m is constant over the year. Geothermal heat exchangers may decrease or increase the temperature locally. Therefore, in our study we focus on the effects of temperature changes on the subsurface environment adjacent to geothermal heat exchangers. Temperature shifts influence the viscosity and density of water but also the solubility of liquids, solids, gases and generally the geochemical equilibrium. Not only may geochemical equilibria shift but also the microbial communities and fauna may be influenced by temperature. While temperature effects on the microbial community for open, nearer surface geothermal systems are documented [1,2] the current project aims to evaluate possible shifts induced by geothermal heat exchangers.
With this study we are presenting first results for the effect of changing temperatures on the microbial community in a usually constant temperature environment such as the subsurface.
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SMP043

Microbial carbon decomposition under anoxic conditions in permafrost-affected soil of the Qinghai-Xizang Plateau

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The Qinghai-Xizang Plateau (QXP) in high Asia is the third permafrost unit outside polar regions, about 54.3% of it is covered by permafrost, retaining 23% SOM of Chinese soils or 2.5% of the global pool (Wang et al., 2002). Affected by India and Asian monsoon, the plateau differs in temperature and moisture gradients along the monsoon routes, making the fate of SOM within QXP soil more complicated. The SOM turnover is mainly driven by microbial communities which decompose permafrost SOM via a sequence of microbial processes to CH_4 under anaerobic conditions (methanogenesis), which is a rather strong greenhouse gas. In response to climate warming, QXP permafrost degradation has been enhanced over the past decades, the methane turnover via methanogens is therefore our focus from the view of global change research. An interdisciplinary project is conducted along the two different monsoon gradients to link thein-situ methane flux with temporal and spatial variations of permafrost soil carbon (e.g. ST, SM, SRP, SOMs, TOC, pH) and dynamics of methanogenic consortia. Attention will be paid on the anaerobic carbon decomposition, dynamics of archaeal communities and their reaction to global change by using methane producing rate analysis and a diverse molecular biotechniques including DGGE and t-RFLP fingerprints, cloning, FISH and real-time PCR to quantitatively and qualitatively investigate the diversity, abundance and the changes of the composition of archaeal communities. On this basis, it is expected to be able to improve our understanding about the potential anoxic decomposition of permafrost SOMs under different the climate gradients and its future development under global warming.
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SMP044

RNase J and RNase E in Sinorhizobium meliloti: specific and common roles in rRNA maturation, RNA modification, motility and quorum sensing

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Sinorhizobium meliloti Rm2011, a nitrogen-fixing plant symbiont, harbours RNase E and RNase J, two principal RNases of Bacillus subtilis and Escherichia coli, respectively (1). To address the mechanisms for posttranscriptional regulation of gene expression inS. meliloti, we analyzed mutants with mini-Tn5 insertions in the genes encoding RNase E and RNase J (2) in comparison to the wild type. Only the RNase J mutant but not the RNase E mutant was impaired in growth, motility and rRNA maturation (3). However, small RNAs, tRNAs and mRNAs were affected in both mutants. Small RNAs and tRNAs have identical lengths but migrate differently in denaturing gels when compared to the wild type, suggesting hypermodification of RNA in the two mutants. Consistent with this, the gene SMc00649 encoding a probable RNA methylase was up-regulated in the mutant strains. Additional microarray and qRT-PCR analyses revealed specific and overlapping effects on mRNA level. The detected down-regulation of genes involved in motility and chemotaxis in the two mutants suggested differences in the quorum-sensing response in comparison to the wild type. Indeed, production of AHLs was increased in the mutant strains, while overproduction of RNase E resulted in a strong decrease of the AHL amounts. Analysis of genes involved in AHLs production showed that balanced expression of RNase E ans RNase J is important for the posttranscriptional control of quorum sensing in S. meliloti.

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SMP045

Change of microbial community composition due to geothermal use of the subsurface

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Seasonal heat storage systems for district heating and building climatisation are of increasing importance to secure a sustainable energy use and supply. For an efficient and permanent reliable use of geothermal energy the impact on the environment has to be evaluated.
Our presentation encompasses a study of a lab-scale column experiment to quantify the effects of different temperatures on solution, precipitation and microbially catalysed redox processes.
Four different tempered columns (10, 25, 40, 70°C) were operated and sodium actetate was added continuously. To characterize the microbial biocenosis of the initial sediment samples, fluid samples from the upper exit and also over the profile (9 sampling ports) were collected. All samples were analysed based on partial 16S rDNA. Among fingerprinting methods (PCR-DGGE) for the characterization of the microbial biocenosis, qPCR and FISH will be applied for the quantification of microorganisms and the determination of their metabolic activity.
Sulfate reduction in all columns was detected with the highest reduction rates at 40°C. First fingerprinting results show a shift of the dominant microorganisms due to the different temperatures. Additionally, the microbial composition in the 10°C column changed clearly in between the different sampling ports of the column. Methane production was measured at 25°C correlating with Archaea occurrence.
Lab-scale column experiments showed an alteration in the microbial biocenosis due to geothermal induced temperature effects. The identification of microorganisms enables the correlation to metabolic classes and provides information about biochemical processes in the used groundwater system and therewith the impact on plant operation as well as environment.

SMP046

Characterization of IncP-9 in different biofilters from Belgium contaminated with pesticides

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Conjugative plasmids seem to be one of the mobile genetic elements most responsible for the rapid adaptation to environmental selective pressure. Consequently, plasmids play a major role in enhancing the genetic diversity and adaptation of bacteria as agents of horizontal gene transfer (HGT). IncP-9 plasmids are very important vehicles for degradation and resistance genes that are assumed to contribute to the adaption of bacterial communities in environments contaminated with xenobiotic compounds such as biofilters that are used for microbial degradation of pesticides. In this study the abundance and diversity of IncP-9 plasmids in six different biofilters (three replicates per biofilter) from Belgium was analyzed.A newly developed primer system targeting therep-oriVregion (S1: 610 bp) was used for PCR amplification from total community DNA. Southern blot hybridization confirmed the presence of IncP-9 plasmid specific sequences in all biofilters with one exception. In order to obtain insights into the diversity of the IncP-9 plasmids, amplicons obtained from biofilters 1 (Leefdaal), 2 (Pcfruit) and 5 (Kortrijk), that showed the strongest IncP-9 signals, were cloned and sequenced. In addition, a quantitative real time PCR system (S2: ~200 bp) was established in order to quantify IncP-9 abundance. The log10 transformed ratio of IncP-9 to16S rRNA gene copies varied from -3.1 to -2.65. To validate the specificity of the qPCR primer system, the amplicons were also cloned and sequenced. Both systems specifically amplified IncP-9 sequences from total community DNA. While all sequences amplified with both primer systems from biofilter 5 showed a high sequence similarity to IncP-9 η (pNL15), sequences obtained from biofilter 1 and biofilter 2 were more diverse, affiliated to the IncP-9a (pMT3 - biofilter 2), IncP-9b (pWW0 - biofilter 1), IncP-9d (pNAH7; only obtained with S1- biofilter 2) and IncP-9 η (pNL15 - biofilters 1 and 2). In addition several novel sequences only distantly affiliated to the clusters defined by Sevastsyanovich et al., 2008 were obtained from biofilters 1 and 2. Interestingly only a small number of identical sequences were picked up with both systems indicating a surprisingly high sequence diversity of IncP-9 plasmids in biofilters.

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SMP047

Minerals and charcoal - factors shaping microbial community composition and bacterial response to phenanthrene in artificial soils

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In soil, different organic, inorganic and biological constituents are contacting each other and forming large biogeochemical interfaces. Their interactions are poorly understood and therefore this study explored the influences of soil minerals and charcoal on microbial communities. Due to problematic comparison of natural soils, in a microcosm experiment seven artificial soils were composed varying in clay minerals (illite, montmorillonite), metal oxides (ferrihydrite, boehmite) and charcoal. The same aliquots of the microbial fraction extracted from Cambisol were used as initial microbial community and autoclaved manure as nutrient source for each artificial soil. Incubation took place under constant environmental conditions up to 18 months (sampling on day 1, 9, 31, 90, 180, 460, 450). Total community DNA was extracted and the 16S rRNA gene and ITS amplicons for *Bacteria* or *Fungi*, respectively, were used in denaturing gradient gel electrophoresis (DGGE) to generate molecular fingerprints. DGGE analysis showed that mineral composition and charcoal influence the establishment of microbial communities in artificial soils, even after a long incubation time. Especially the charcoal soil showed pronounced differences in the DGGE pattern compared to other artificial soils without charcoal.
To explore the response of the established microbial communities to persistent organic pollutants, one-year old artificial soils were spiked with phenanthrene (2 g/kg) and incubated for another 70 days. By DGGE, shifts in the bacterial but not fungal communities were revealed between non-spiked and phenanthrene-contaminated samples. Interestingly, bacterial communities of different artificial soils showed distinct phenanthrene responses. By plating, higher bacterial counts were found in soils treated with phenanthrene.
In conclusion, minerals and charcoal in artificial soils shaped the composition of microbial communities and the bacterial response to phenanthrene.

SMP048

Interactions between bacteria antagonistic towards *Rhizoctonia solani*, lettuce and indigenous rhizosphere communities in three soil types

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Rhizoctonia solani is a soil-borne plant pathogen which causes bottom rot disease and leads to a massive loss of lettuce and potato every year. The lack of effective fungicides makes it difficult to control this and other plant pathogens. So it is necessary to find alternative strategies. A promising approach is the use of natural antagonists of the plant pathogen. Under laboratory and greenhouse conditions, two isolates, *Pseudomonas jessenii* RU47 and *Serratia plymuthica* 3Re4-18, showed the ability to reduce disease symptoms. But the efficiency of biocontrol agents was reported as very variable and the reason for this variability is largely unknown. Therefore, a better understanding of the interaction of the microbial community, the plant rhizosphere and the bulk soil is required for a successful exploitation of this antagonistic potential. Within the frame of a DFG-Project a field experiment has been set up with a unique experimental plot system in Großbeeren comparing three different soil types. This made it possible to analyze the influence of the soil type independently from other factors such as climate and cropping history. First results showed a different survival of the two antagonistic strains in the tree soil types. Also the damages caused by the pathogen are different in the soil types. We assume that these observations are related with differences in the microbial communities of the three soils. Indeed highly significant differences between the soil types were revealed by denaturing gradient gel electrophoresis analysis of bacterial and fungal communities. Looking at the rhizosphere, it could be confirmed that after three weeks, when lettuce is especially susceptible to the pathogen, the antagonistic strains are dominant populations. The antagonists compensated the damage caused by the pathogen similarly in all soils as revealed by dry weight and rating of the lettuce. Also the antagonistic strains did not affect the indigenous microbial community. Therefore a negative ecological effect is not expected. The antagonistic strains had a positive influence on lettuce independent from the indigenous microbial community and the pathogen so the biological mechanism remains unknown. In conclusion the strains are promising biocontrol agents to compensate the lack of effective fungicides.

SMP049

Effects of resource quality and quantity on fungal communities in an agricultural soil

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Due to their high diversity and a wide decomposition potential, fungi are well adapted to the heterogeneous soil environment and are a major component of soil microbial communities.
In the frame of the DFG-funded (German Research Foundation) research unit FOR 918 „Carbon flow in belowground food webs assessed by isotope tracers“ we investigate the role of saprobiotic fungi in the transfer of organic carbon from plant origin to belowground food webs of an agricultural soil. To tackle how carbon quality and availability influence the fungal communities, a field experiment has been installed where two crops, maize (Zea mays L.) und wheat (Triticum aestivum L.), are cultivated in a design cross manipulating addition of maize litter. Soil from three depths was sampled in July, September and December in 2009 and 2010 to analyze seasonal shifts in the fungal community composition.
ARISA (automated ribosomal intergenic spacer analysis) which was used as DNA-fingerprint method resulted in 198 OTUs (operational taxonomic units). Univariate statistical analysis revealed that fungal species richness varies according to the crop and the manipulated carbon input in terms of added litter. Furthermore fungal species richness was highest in September in both years. Despite a reduced carbon availability in the B-horizon no decline in species richness with increasing depth was found.
Multivariate statistical analysis demonstrated that the soil fungal community is mostly affected by soil depth, followed by the impact of the plants and related root exudates. These results indicate strong reactions of the fungi to different nutrient supplies.
In follow up microcosm experiments with variable nutrient availability fungal key players actively assimilating carbon will be identified using rRNA-SIP (stable isotope probing).

SMP050

Minimal nutrient requirements of *Myxococcus xanthus* DK1622

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The soil bacterium *Myxococcus xanthus* DK1622 naturally feeds on lysed microorganisms by secretion of proteases [1, 2]. Since the growth is poor on defined media like A1 or M1 [3, 4, 5], but good on casein and casein hydrolysates, we studied the degradation and uptake of related peptides. Its lack of a hexose uptake system [1] does not allow utilizing mono- or polysaccharides. Isoleucine, leucine and valine are essential because they cannot be synthesized [3], but their uptake is enabled by the branched chain amino acid transport system [6].
Like in the degradation of β-casein by *Lactococcus lactis* [7], uptake of peptides should be possible with a maximal length of 18 amino acids via the oligopeptide permease [8]. The biological fate of peptides in the culture supernatant of *M. xanthus* was followed via Matrix-assisted laser desorption/ionisation mass spectrometry (Maldi-MS) with bradykinin 1-7 as internal standard (any occurrence of m/z=757 in supernatant). The sequences of single peptides were confirmed by tandem measurements (MS/MS) with collision induced decay and a novel software tool (PeptideChopper 2.0) which aligns the found masses of peptides and fragment ions to all four casein subtype sequences.
Main degradation detectable by Maldi-MS takes place at β-casein 59-92 and 144-162. We identified main and alternative degradation pathways by both C- and N-terminal exopeptidases. A kinetic model was developed to describe the degradation of peptides.
To observe whether uptake of peptides with a length of nine amino acids is possible without further degradation, synthesized β-casein peptides 74-82 and 145-153 which contain all amino acids essential for growth were incubated with concentrated proteases from the culture supernatant to find the putative degradation products. In a second experiment, they were used as the only carbon and nitrogen sources.
This way, we elucidated degradation pathways of β-casein by *M. xanthus* DK1622. Offering a high amount of synthetic peptides containing all essential amino acids is not sufficient for efficient growth of *M. xanthus* DK1622 even if they are parts of the major degradation pathways of casitone.

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SMP051

Insights in anaerobic hydrocarbon biodegradation under methanogenic conditions

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Enrichment, isolation and characterisation of hydrocarbon degrading microorganisms are of great importance to understand the biochemical mechanisms responsible for oil biodegradation in contaminated environments and in petroleum reservoirs. With respect to decreasing conventional energy resources this understanding also helps in the search for methods of enhanced hydrocarbon recovery, like the microbial conversion of oil or coal to recoverable methane.
The main focus of this work therefore is the biodiversity of hydrocarbon degraders and their metabolic processes of methanogenesis. We started to investigate the physiological characteristics and activities of microbial consortia enriched from freshwater and marine sediments as well as from oil and coal reservoirs.
Stable isotope measurements showed the conversion of ^{13}C-labelled hydrocarbons into methane. With the use of T-RFLP and Q-PCR a large bacterial diversity was detected while the archaeal was limited to three or four dominant species. Both domains were highly abundant in all enrichment cultures. Genes indicative of metal reduction, sulphate reduction, and methanogenesis were also detected in high numbers in these incubations. The sequencing analysis revealed a low phylogenetic diversity of *Archaea* comprised of *Euryarchaeota* and *Crenarchaeota*. Members of *Methanosarcinales* and *Methanomicrobiales* dominated the archaeal part of the community in the enrichment cultures. The main bacterial representatives were *Syntrophus* spp., *Desulfovibrio* spp. and *Syntrophomonas* spp.. Using stable isotope probing with different ^{13}C-

labelled hydrocarbons or potential intermediates of the methanogenic degradation pathway, combined with molecular and biochemical analyses, we are attempting to reveal the carbon flow as well as the active microbial community in the enrichment cultures.

SSV001

Metabolic pathway fluxes of the marine model bacterium *Dinoroseobacter shibae* under changing environmental conditions

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The *Roseobacter* clade is one of the most prevalent bacteria in marine habitats and is living in various ecological niches [1]. As indicated from recently sequenced genomes, they comprise a rich repertoire of metabolic pathways [2, 3]. *Dinoroseobacter shibae* as prominent member of the Roseobacterclade is additionally known for its ability to grow in a symbiotic relationship with algae, to produce acetylated homoserine lactones (AHL), and to perform aerobic anoxygenic photosynthesis. First studies of the central carbon metabolism showed that glucose is metabolized exclusively via the Entner-Doudoroff pathway [3]. This unusual flux distribution differs from most terrestrial microorganisms [3, 4]. For a more detailed view into the carbon core metabolism of *D. shibae*, state of art ^{13}C metabolic flux analysis was applied [5]. This comprised the creation of a metabolic network model from available pathway information (databases from Kyoto Encyclopedia of Genes and Genomes and Joint Genome Institute). The model was then integrated into the modelling software platform OpenFLUX [6]. For the first time, this allowed to investigate the physiological response of *D. shibae* on the flux level to changes in environmental conditions such as nutrient status, temperature or salt level, providing a first systems level insight into this important marine model organism. In conclusion fluxes remained quite unaffected by environmental perturbation, which indicates a distinct homeostasis as well as a high robustness of *D.shibae*. This might partly explain the enormous success of this bacteria and its related species in the marine realm.

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SSV002

Glucosyl-glycerate is a nitrogen stress-dependent carbon-capacitator in *Mycobacterium smegmatis*

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Question: Nutrient depletion often requires an organism to drastically alter its physiology and metabolism. We investigated the response to nutrient depletion in the form of nitrogen starvation of the bacterium *Mycobacterium smegmatis*, an important model for the study of the human pathogen *M. tuberculosis*.
Methods: We profiled the metabolic response of *M. smegmatis* to nitrogen starvation, by quantifying the changes in exo- and endometabolome over time using NMR spectroscopy as well as mass spectrometry. Additionally, we replenished nitrogen and quantified the metabolic consequences of this nitrogen up-shift.
Results: Interestingly, cells of *M. smegmatis* continued to divide and grow after the extracellular nitrogen source is depleted (albeit at a slower rate) hinting at the presence of an intracellular storage molecule. Concomitant with extracellular nitrogen run-out, levels of glycerone showed a transient increase. Inside the cells, low nitrogen triggers the accumulation of glycogen and other carbon storage molecules including the disaccharide trehalose and the hexose-conjugate glycosyl-glycerate (GGA), which accumulates to high (approx. 500 mM) concentrations inside the cytosol.
Following nitrogen up-shift, the metabolism of the cells was drastically altered, leading to a sharp increase in glutamate and trans-aconitate. This coincided with a decrease in GGA. Interestingly, a mutant unable to synthesise GGA is not viable in low nitrogen concentrations despite the molecule itself not containing any nitrogen.
Conclusion: Our study shows that the mycobacterial responses to nitrogen starvation are not yet fully understood, and potentially involve novel metabolic regulation. We found that extracellular nitrogen availability controls intracellular carbon turnover, but surprisingly is not an absolute prerequisite for growth. Instead, the ability to synthesise a carbon storage molecule that accumulates during nitrogen shortage is essential for growth in low nitrogen concentrations.

SSV003

Flavohemoprotein Hmp of *Corynebacterium glutamicum* is involved in nitrosative stress resistance

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Corynebacterium glutamicum is a Gram-positive soil bacterium, which is used in industrial biotechnology for the production of amino acids [1]. Only recently it was discovered that it can also grow under anaerobic conditions by means of nitrate respiration [2,3]. In this process nitrite is formed, which cannot be reduced further by *C. glutamicum* and therefore accumulates in the medium. Nitrate respiration and the presence of nitrite can trigger the formation of reactive nitrogen species, which are toxic for the cell. Hence, nitrosative stress tolerance has become of interest in order to improve anaerobic growth of *C. glutamicum*. We could show that nitrite inhibited aerobic growth of *C. glutamicum* in a concentration-dependent manner. The NO-donating agent sodium nitroprusside (SNP) also decelerated aerobic growth. Studies on the impact of nitrite on global gene expression under aerobic conditions revealed that the gene cg3141 (*hmp*) was 10-fold upregulated. In other organisms, e.g. *E. coli*, flavohemoprotein Hmp has been shown to mediate resistance towards nitric oxide [4]. Deletion of *hmp* in *C. glutamicum* ATCC13032 resulted in a strain (Δ*hmp*) which is more sensitive towards nitrite and SNP than the wild type. This phenotype was complemented successfully by plasmid-based expression of *hmp*. Anaerobic growth with nitrate of the Δ*hmp* mutant was also retarded in comparison to the wild type. These results demonstrate that the flavohemoprotein Hmp of *C. glutamicum* is important for nitrosative stress tolerance under aerobic and anaerobic conditions.

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SSV004

Drug efflux as a surviving strategy in response to the anaerobic stress in E. coli

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Multidrug efflux pumps are well known for their ability of removing intracellular antibiotics from bacteria and causing antibiotic and multidrug resistance during the infectious diseases treatment. Bioinformatics and genome-wide studies have revealed that efflux genes indeed are widely distributed in all living organisms and constitute from 6% to 18% of all transporters in bacterial genomes, suggesting a more general role of this class of gene products in bacterial physiology beyond just causing antibiotic resistance. In pursue of these physiological functions especially during the process of bacterial stress response, we examined the expression of all 20 efflux systems encoded in E. coli genome under the anaerobic stress conditions. This led to the identification of a dramatic up-regulation of an efflux pump, MdtEF, under this condition, which is independent of antibiotic exposure. Expression of MdtEF is found to be up-regulated more than 20 fold by the global regulator ArcA under anaerobic conditions, resulting in increased efflux activity and enhanced drug tolerance inE. coli under this condition. To explore physiological functions of MdtEF, we constructed ΔmdtEF strain and found that E. coli K-12 cells lacking the MdtEF efflux pump display a significantly decreased survival rate when cells reduce nitrate via anaerobic respiration. Replacing nitrate with fumarate as the terminal electron acceptor, or deletion of the genes tnaAB which are responsible for the biosynthesis of indole, restores the viability of the ΔmdtEF strain under anaerobic respiratory conditions. Further investigation revealed that cells lacking the MdtEF efflux pump are susceptible to indole nitrosated compounds, a class of toxic by-products which are formed and accumulated during the nitrate respiration in E. coli owing to the generation of reactive nitrogen species (RNS) under this condition. Taken together, we propose that E. coli activates the multidrug efflux pump MdtEF to remove the toxic nitrosated indole derivatives during its anaerobic respiration of nitrate, thus providing a surviving strategy against nitrosative damages during its lifestyle in the anaerobic ecological niches.

SSV005

Metabolic adaptation of *Acinetobacter* to changing environmental conditions

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Members of the genus *Acinetobacter* are metabolic versatile, ubiquitous organisms occuring in soil and aquatic ecosystems but many have also been recovered from human clinical specimens. Persistence of *Acinetobacter* strains in their environments does not only involve the ability to find nutrients, but also to cope with physiochemical changes. Among those are changes in water availability as, for example, caused by salinity or desiccation. In previous studies we could already show that *A. baylyi* can cope with high salinities by uptake and accumulation of the well known compatible solute glycine betaine (Sand *et al*. 2011)[1].
Here we have adressed the question whether *A. baylyi* can use choline as precursor for glycine betaine synthesis in order to adapt to high osmolarities. In *A. baylyi* the uptake of choline was found to depend on the presence of choline in the growth medium, but not on high salinities. At high salinities choline was accumulated in the cells and oxidized to glycine betaine whereas in the absence of osmotic stress choline was taken up, oxidized and subsequently exported out of the cells. Inspection of the genome sequence revealed a bet-cluster comprising of two genes for putative choline transporters (*ACIAD1011, betT*), one regulator gene (*betI*), and two genes encoding dehydrogenases for the oxidation of choline to glycine betaine (*betA, betB*). Mutant studies, choline transport and oxidation studies as well as transcriptional analyses of the bet genes led to the identification of two distinct choline transporters: an osmoregulated and a salt-independent transporter. Both, the structural genes for choline oxidation and the choline transporter genes undergo transcriptional regulation by BetI.

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SSV006

The incompatible solute creatine inhibits bacterial Na^+/H^+ antiporters

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The accumulation of compatible solutes (organic osmolytes) from the environment is an organism´s prime rapid stress response against increased salinity (osmolarity). In previous studies it has been shown that this response can go seriously wrong when structurally related but inhibitory compounds (named incompatible solutes) are "mistaken" for the most common compatible solutes glycine betaine and ectoine [1]. Such inhibitory compounds are the naturally occuring creatine and the synthetic ectoine derivative guanidino-ectoine, both of which are characterized by a guanidinium moiety [2].
Since 2-aminoperimidine (a guanidinium-containing naphthalene derivative) was shown to act as specific inhibitor of NhaA-type Na^+/H^+ antiporters from Escherichia coli [3], we investigated the effect of the above incompatible solutes on the activity of such antiporters. Inside-out membrane vesicles of E. coli K-12 were used to measure the antiport activity with the help of acridine orange, a fluorescent probe for pH difference across the membrane.
Thus we were able to demonstrate that creatine clearly inhibits Nha activity at μM concentrations while neither its corresponding compatible solutes betaine nor ectoine-type compatible solutes affected antiport activity. The fact that creatine is actively accumulated into the cytoplasm distinguishes it from other inhibitors of Na^+/H^+ antiporters (so far only investigated at inside-out vesicles) and opens up potential applications as selective growth-inhibitory compound for a range of Gram-negative bacteria (exhibiting osmolyte uptake systems and Nha-type antiporters).
Although the guanidino group is believed to be the critical function for its inhibitory effect on sodium-proton antiporters, mimicking a tri-hydrated sodium ion, we must, however, conclude from our studies that guanidino-ectoine has a different mode of action.

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SSV007

How *Cupriavidus metallidurans* deals with toxic transition metals

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Cupriavidus metallidurans is one of the model organisms for understanding metal homeostasis in heavy metal containing environments. Toxic-only metal cations such as Cd^{2+} (with one beneficial exception) are removed from the cytoplasm by metal efflux when the concentration of such a cation increases above a threshold. In contrast, an intriguing metal homeostasis system has to keep the concentration of essential-but-also-toxic cations such as Zn^{2+}, Co^{2+} and Ni^{2+} in the cytoplasm in the correct albeit narrow range. Homeostasis is achieved by metal-binding reactions based upon a thermodynamical flow equilibrium of metal uptake and efflux reaction. To define a standard, we measured first how many metal atoms are present in a *C. metallidurans* cell after growth in mineral salts medium. The investigation of the metal content inside the cells revealed no change in the Zink content but a nearly 12fold lower concentration of manganese in *C. metallidurans* than in *E. coli* probably due to the absence of an NRAMP uptake system for manganese and the absence of a manganese dependent superoxide dismutase. The orchestra of metal efflux systems in *C. metallidurans* has been investigated in details in the past and comprises a set of RND-driven transmembrane protein complexes that transport cations from the periplasm to the outside plus primary exporters of the P-type ATPase protein family and secondary transporters from various protein families. These could be assigned to central substrates, e.g. DmeF to cobalt, CadA to cadmium, ZntA to zinc, PbrA to lead, FieF to iron and CnrT to nickel. Additionally, an in-depth characterization of the metal uptake systems by stepwise multiple deletion was carried out, including the genes for the following transporters: ZupT of the ZRT/IRT, PitA of the phosphate inorganic transporter, four CorA paralogs of the MIT, HoxN of the NiCoTprotein family and two P-type ATPases. All of these seem to transport a wide range of metal cations including Zn^{2+}. In contrast to the exporters, these importers could not be assigned to central substrates. Thus, metal homeostasis in *C. metallidurans* is achieved by highly redundant metal uptake systems, which have only minimal cation selectivy and are in combination with metal efflux systems that “worry later” about surplus cations.

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SSV008

Accept your fate? Defence strategies of yeast and filamentous fungi against the chitin synthase inhibitor AFP

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The emergence and spread of pathogenic bacteria and fungi that are resistant to virtually all available antimicrobials represents a serious challenge for medicine and agriculture and has stepped up efforts to develop new antimicrobials. The use of smarter antibiotics, also called“dirty drugs”affecting multiple cellular targets is one discussed strategy to prevent the development of resistance mechanisms. Of special interest is the exploitation of antimicrobial peptides (AMPs), which are natural products of pro- and eukaryotic organisms and function as defense molecules to combat nutrient competitors, colonizers or invaders. The activities of signaling pathways are critical for fungi to survive antifungal attack and to maintain cell integrity.However, little is known about how fungi respond to antifungals, particularly if these interact with multiple cellular targets.
The antifungal protein AFP is a very potent inhibitor of chitin synthesis and membrane integrity in filamentous fungi and has so far not been reported to interfere with the viability of yeast strains. With the hypothesis that the susceptibility of fungi toward AFP is not merely dependent on the presence of an AFP-specific target at the cell surface but relies also on the cell’s capacity to counteract AFP, we used a genetic approach to decipher defense strategies of the naturally AFP-resistant strain Saccharomyces cerevisiae.The screening of selected strains from the yeast genomic deletion collection for AFP-sensitive phenotypes revealed that a concerted action of four signalling pathways is likely to safeguard *S. cerevisiae* against AFP. Our studies uncovered that the yeast cell wall gets fortified with chitin to defend against AFP and that this response is largely dependent on calcium/Crz1p signaling. Most importantly, we observed that stimulation of chitin synthesis is characteristic for AFP-resistant fungi but not for AFP-sensitive fungi, suggesting that this response is a successful strategy to protect against AFP. We thus propose the adoption of the damage-response framework of microbial pathogenesis for the interactions of antimicrobial drugs and microorganisms in order to comprehensively understand the outcome of antimicrobial treatments.

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SSV009

Mathematical modelling of cooperation and cheating in survival strategies of microorganisms

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Microorganisms are often engineered to produce a extracellular enzymes, for example, for producing renewable fuels and in biodegradation of xenobiotics. Productivity is often reduced by "cheater" mutants, which are deficient in exoenzyme production and benefitting from the product provided by the "cooperating" cells. The interplay between cooperation and cheating can be described theoretically by game theory [1,2]. We present a game-theoretical model to analyze population structure and exoenzyme productivity [3]. Three distinct regimes are predicted: when the metabolic effort for exoenzyme production and secretion is low, all cells cooperate; at intermediate metabolic costs, cooperators and cheaters coexist, while at high costs, all cells use the cheating strategy [2-4]. These regimes correspond to the harmony game, snowdrift game, and Prisoner's Dilemma, respectively. Thus, microbial strains engineered for exoenzyme production will not normally be outcompeted by cheater mutants. Moreover, our model provides an estimate of the cell density maximizing exoenzyme production [3].
Another example of different survival strategies that can be modelled by game theory concerns the polymorphic fungus *Candida albicans*. Two strategies are available for each pathogenic yeast cell once being phagocytosed: avoiding lysis transiently (called silencing here) or forming hyphae and escaping (called piercing). Two different outcomes can be derived from our model: when the difference of the costs of the two strategies is low, all fungal cells inside a macrophage will play the piercing strategy, while in the high-cost case, piercing and silencing cellscan coexist [5]. Further, the role of theSAPgene family encoding secreted proteinases and the Sap proteins is investigated and is put in relation to the costs of the strategies. Our results are in agreement with wet-lab results presented by other groups and the model parameters can be estimated from experimental data.

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SSV010

Staphylococcus aureus persister cells tolerant to bactericidal antibiotics

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Bacterial cultures contain a subpopulation of persister cells, non- or slow growing reversible phenotypic variants of the wild type, tolerant to bactericidal antibiotics. To establish parameters for selection of *Staphylococcus aureus* persisters, we monitored CFU counts of planktonically grown cells treated with a number of antibiotics over time. Strains SA113, HG001, HG002, and HG003 and small colony variants (SCVs) *hemB* and *menD* were challenged by the drugs added at different logs of MIC in exponential- or stationary growth phase. Generally, antibiotic tolerance was elevated in SCV strains compared to normally growing cells and in stationary vs. exponential growth phase, but biphasic killing kinetics, typical for persister cell selection, were observed in both types of cultures. Treatment of strains HG001-HG003 in exponential phase with 10-fold the MIC of tobramycin resulted in the selection for both persisters and phenotypically stable SCVs. Trajectories of different killing curves indicated physiological heterogeneity of fitness within a pool of persisters. 100-fold MIC of daptomycin added to stationary phase SA113 cells rapidly selected for very robust persisters. Although cells pretreated with an antibiotic exhibited elevated tolerance upon immediate reexposure to the same drug, the persister state was not vertically transmitted. A shift of persisters to non-selective media evoked resuscitation and resumption of growth after about three hours. Our data provide insights into persister dynamics and reveal important roles of growth phase, strain background and genotype on persister levels of *S. aureus*.

SSV011

Sodium hypochlorite stimulates c-di-GMP synthesis and biofilm formation in *Pseudomonas aeruginosa*

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The Gram-negative bacterium *Pseudomonas aeruginosa* plays an important role as an opportunistic pathogen in infectious diseases. Due to the size and complexity of its genome as well as the sophisticated and coordinated regulation of gene expression mediated by a large number of regulatory elements, *P. aeruginosa* is able to adapt to various environments. One important strategy in order to survive stressful environmental conditions e.g. growth in the presence of antimicrobial agents such as antibiotics or biocides is the formation of resistant biofilms. To investigate the stress response of *P. aeruginosa* PAO1 to sodium hypochlorite, a disinfectant which is commonly used in hospitals and drinking water treatment, we analyzed bacterial growth and biofilm formation in the presence of free chlorine at different concentrations. In static biofilm assays, free chlorine at subinhibitory concentrations led to a two-fold increase in attachment after two hours of incubation compared to the non-treated controls. The altered biofilm structure caused by sodium hypochlorite treatment was further studied by fluorescence microscopy. Microarray analyses of chlorine treated cells compared to untreated controls revealed a significant upregulation in the expression of different genes which are known to be involved in attachment and biofilm formation, e.g. genes implicated in type IV-pili, flagella and alginate biosynthesis and function. Moreover we found an enhanced expression of ORF PA3177 which codes for a putative di-guanylate-cyclase. Di-guanylate-cyclases catalyze the synthesis of the second messenger c-di-GMP which is an important factor in biofilm formation and persistence in *P. aeruginosa*. Subsequent LC-MS/MS analyses of bacterial lysates showed indeed a more than 5-fold increase in c-di-GMP levels in response to chlorine treatment suggesting a key role of this second messenger in chlorine induced biofilm formation. The function of PA3177 in the stress response of *P. aeruginosa* towards biocides was further investigated in more detail.

SSV012

SiaABCD, a signaling pathway controlling autoaggregation in *Pseudomonas aeruginosa*

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The formation of biofilms and cell aggregates is environmentally responsive, often proceeding in a sequential manner involving complex regulatory mechanisms. The lack of knowledge regarding environmental stimuli and the apparent redundancy of pathways leading to biofilm formation provide challenges for studying its genetic regulation.
Previously, we showed that *Pseudomonas aeruginosa* forms freely floating aggregates as an adaptive survival strategy in the presence of the toxic surfactant sodium dodecyl-sulfate(SDS). While aggregate formation was triggered by SDS exposure, it was not a prerequisite for survival under growth permissive conditions, making this a suitable model system to analyze the molecular mechanisms involved in aggregate formation. We found that expression of the adhesive fimbriae CupA and the extracellular polysaccharide Psl is essential for autoaggregation in response to SDS stress and that this phenotype is regulated by the novel signaling pathway SiaABCD.
We now report that the gene PA4623, located immediately upstream of the previously described two-partner secretion system encoded by *cdrAB*, is essential for SDS-induced aggregate formation. This is in agreement with previous microarray data showing increased expression of PA4623 and *cdrAB*, exclusively for aggregated cells during SDS exposure. To further investigate the molecular mechanisms of SDS-induced aggregation, we performed a systematic mutational analysis of the *siaABCD* operon and characterized the corresponding mutants. While a *siaB* deletion promoted autoaggregation, a non-functional *siaC* allele completely abolished aggregation during SDS exposure. Interestingly, strains overexpressing *siaB* generally exhibited a non-aggregative phenotype, indicating an important regulatory function of the SiaB protein. Further, secondary mutation analysis suggest that the SiaABCD pathway may operate via a bi-functional mechanism, involving c-di-GMP signaling and the regulation of mRNA stability.

SSP001

Microbial species show adaption for survival in adverse medium by inducing changes to glycosidic products before and during the sporulation stage.

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Most microbial species have a life cycle pathway that involves continuous adaption of the nutrients in the surrounding media.This depends on the immediate needs of the organism at any particular instant in the life cycle process.Many microbial species use the method of changing glycosidic and polysaccharidic related products both inside the organism and in the surrounding milieu to their needs at any particular point in the pathway.These changes are made by varying the structure of glycosidic products and the polymeric length of products according to the basic

chemical constituents are avaliable.This adaption continues to the stage of sporulation where the structure and size of the sporulating entity is vital to the next stage in the life cycle pathway.Two species that show this adaption well are xanthomonas and clostridia in the strains xanthomonas campestris pv campestris and clostridia felsineum pv felsineum.It can be seen both in structural analysis of the pathway products of these two examples by various methods how this adaption occurs.This process of adaption gives a deep insight into the microbial organisms self modification within the life cycle pathway.

SSP002

Membrane-active antimicrobial peptides can trigger the transition of bacteria into a dormant stage

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Question: One of the survival strategies of bacterial populations is the production of a small number of dormant persister cells that grow in a form of small-colony variants (SCVs) [1]. These are tolerant to antibiotics and represent an attached bacterial growth in microbiofilm mode. The transition into the dormant stage occurs during the SOS response and is modulated in *E.coli* by the membrane associated TisB peptide, which decreases proton motive force and ATP biosynthesis [2]. We observed that antimicrobial peptides (AMPs), which interact with the bacterial plasma membrane and perturb its integrity [3], can have a similar effect as TisB and lead to the generation of undesirable persister cells. Since these cells reduce their metabolic activity, we suggest that cell respiration may serve as a possible indicator of the transition of cells into the dormant stage.
Method: We developed a microdilution alamarBlue™ respiration assay for monitoring the Bacterial Respiration (BR) as a cellular response to unfavourable conditions. The respiration activity of four bacterial strains was measured as a difference in the absorption between reduced and oxidized forms of the redox indicator alamarBlue™ during 3 hours after inoculation. The microtiter plates were then incubated for 24 hours at 37°C to evaluate the ability of cells to grow.
Results: By monitoring of BR, we observed three different kinds of respiration activity with respect to bacterial growth: activation, correlation, or shutdown, - depending on the combination of bacteria and AMP. Shutdown of initial BR at sub-MIC and subsequent growth at higher peptide concentrations was observed for *S.aureus* upon exposure to MAP, PGLa, Magainin and a combination of PGLa/Magainin. In those cases, single cells of *S.aureus* were found to survive and form SCVs, which were visible by magnification and were detectable via dye reduction.
Conclusion: These findings suggest that exposure of *S.aureus* to these membrane-active AMPs induces a transition of single cells into the dormant stage, which can enhance risk of persistent infections.

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SSP003

About a mechanism of stress dependent enzyme activity regulation via interaction with nucleic acids - salt dependent GG-synthesis in *Synechocystis* sp. PCC 6803

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Under osmotic stress the synthesis and accumulation of the compatible solute glucosylglycerol in the cyanobacterium *Synechocystis* sp. PCC 6803 is facilitated by the activation and fine tuning of the key enzyme of the pathway the Glucosylglycerole phosphate synthase (GgpS). At low salt concentrations GgpS is inhibited via an electrostatic interaction with nucleic acids (Novak *et. al* 2010). Upon a sudden increase of the salt concentration GgpS is liberated and present in its active form. During salt acclimation the largest fraction of GgpS is rebound by nucleic acids while the remaining active molecules ensure the ongoing GG-Synthesis according to the external salt concentration and growth rate.
Biotinylation assays revealed the possible binding site for nucleic acids an α-helix near the active centre of GgpS. Four positively charged amino acids might contribute to the interaction, since an exchanged of these amino acids against alanine caused a reduced affinity for the binding and had an impact on enzyme activity. We found that binding to nucleic acids leads to a conformational change of the protein and characterised the binding and inhibition kinetics.
Additionally, we compared regulatory features of GgpS enzymes or similar trehalose phosphate synthases (TPS) originating from organisms adapted to different environments with respect to fluctuations of the salt concentration. We found proteins that do or do not bind to nucleic acids and are or are not inhibited accordingly. The structural differences and the impact of the regulatory circuit of osmolyte synthesis will be discussed.

SSP004

MscCG of *Corynebacterium glutamicum* - a mechanosensitive channel with dual function in osmotic stress response and glutamate production

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Corynebacterium glutamicum is a gram-positive, biotin auxotroph and apathogenic soil bacterium with exceptional importance for the industrial production of various amino acids, especially L-glutamate. The mechanism of glutamate export is not fully understood so far, although *C. glutamicum* has been used for the industrial production of amino acids for decades. Glutamate excretion can be induced by several different treatments, all altering the cell envelope. Recently, evidence was provided that the small MS channel protein MscCG of *C. glutamicum* is linked to glutamate excretion under glutamate production conditions. MscCG belongs to the MscS-type family of mechanosensitive channels, functioning as emergency valves upon an osmotic downshift. The protein is a close relative of the mechanosensitive channel MscS from *E. coli* (286 AA) concerning its N-terminal and pore region. In addition, MscCG carries a long C-terminal domain of 247 amino acids including a fourth transmembrane domain. The electrophysiological analysis of MscCG showed the typical pressure dependent gating behavior of a stretch-activated channel with a current/voltage dependence indicating a strongly rectifying behavior. To unravel the dual function of MscCG as a mechanosensitive channel and as a glutamate exporter, the well characterized E. coli homolog of MscCG, MscS, was used and expressed in a ΔmscCG strain. We also generated selected C-terminal truncations of MscCG in *C. glutamicum*, gain-of-function and loss-of-function constructs of both *E. coli* MscS and C. *glutamicum* MscCG, as well as fusion constructs of these two proteins, and we have investigated the properties of these constructs with respect to mechanosensitive efflux, electrical conductance, gating properties, as well as glutamate excretion. Various recombinant forms of MscCG were shown to be closely similar with respect to conductance, but we found significantly differences concerning glutamate excretion. The results of these experiments argue for MscCG being both a relevant mechanosensitive channel in *C. glutamicum* upon hypoosmotic stress as well as the major efflux pathway for glutamate excretion in response to particular physiologic conditions. Moreover, the results obtained indicate the C-terminal domain of MscCG being of significant impact for function and/or regulation of MscCG activity.

SSP005

Insights into biofilm formation by initial proteome analysis of a novel Antarctic haloarchaeal isolate

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The formation of biofilms initiated by the adhesion to surfaces turns out to be the most dominant mode of life of microorganisms in nature. The screening and characterisation of various haloarchaea strains and isolates demonstrated that the ability for adhesion is widely distributed within the genera Halobacterium salinarumand Haloferax. Further characterisation showed that the adhesive strains were able to form biofilms. Based on this observation we tested seven novel isolates from water samples of a *hypersaline* Antarctic deep lake by a fluorescence-based assay for adhesion. In comparison to the other haloarchaea the novel Antarctic isolate DL24 showed the highest adhesion signal and was chosen for more detailed analyses. Isolate DL24 is a rod shaped, motile, extremely halophilic archaeon, representing a new genus of the Halobacteriaceae.The cells are able to adhere on glass and plastic surfaces forming biofilms of dense packed multi cell layers with towering macrocolonies up to 50 µm in height. Extra polymeric substances (EPS) were composed of free nucleic acids and glycoconjugates.
To identify factors involved in adhesion a proteome analyses of whole protein fractions were carried out by mass spectrometry. By comparative analysis 220 proteins were exclusively identified in the protein fraction of adherent cells. Among these were transcriptional regulators, different factors of two-component systems, a *glycosyltransferase*, a surface glycoprotein as well as proteins required for a functional type IV pili system. The largest group of proteins corresponded to transport systems such as sugar, ion and multidrug transporters. In addition proteins demonstrating a transition from aerob to anaerobic energy conversion were found. This initial proteome analyses showed distinct differences between the adherent and planktonic lifestyle belong to environmental response, transcriptional differentiation, adhesion and biofilm maturation.

SSP006

Initial proteome analysis of a novel Antarctic haloarchaeal biofilm

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Although biofilm formation is the predominant modus vivendi of microorganisms in nature it is only poorly characterized in terms of archaea. It was recently shown that several haloarchaea are able to adhere to surfaces and form biofilms. Since the underlying mechanisms are still unknown we used a proteomic approach for an initial investigation of factors involved in biofilm formation of the novel Antarctic isolate t-ADL DL24 which is able to form densely packed multilayer biofilms.
Static liquid cultures of t-ADL DL24 were cultivated for 28 days in petri dishes before planktonic and adherent cells were harvested separately. Cytoplasmic and membrane fractions were separated by sedimentation. Comparative protein analysis using SDS-PAGE yielded differential protein patterns for planktonic and adherent cells. MS-analyses were performed to identify the protein sets of both phenotypes. Altogether 801 different proteins were identified in cytoplasmic and membrane fractions of adherent cells, corresponding to 23 % of the predicted ORFs, whereas a total of 678 proteins were detected in planktonic cells (20 %). While an overlap of 573 proteins was found in both phenotypes, 228 Proteins were solely detected in adherent cells and 105 proteins were associated with planktonic lifestyle. Categorisation of the phenotype specific proteins according to their cluster of orthologous groups of proteins (COG) provided first insights into the biological processes contributing to biofilm formation of t-ADL DL24. Evidence for an adjustment of energy metabolism in biofilm cells was found, especially proteins indicating a changeover from aerobic to anaerobic energy conversion and multiple transport proteins. Concerning information processing a couple of transcriptional activators as well as components of signal transduction systems and stress responses were exclusively detected in adherent cells. Furthermore a number of biofilm specific proteins related to cellular processes like synthesis of the cell envelope, type IV pili and biofilm matrix were identified.
The results of this first proteome analysis demonstrate that the biofilm lifestyle goes along with fundamental cellular rearrangements on the protein level affecting diverse biological processes in haloarchaea.

SSP007

The role of myo-inositol-1-phosphate synthase in the adaptation of the thermoalkaliphile *Caldalkalibacillus thermarum* to supraoptimal temperature

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Microorganisms encounter rapid and dramatic changes in their environment on a regular basis. One of the most likely and threatening of these changes is an increase in temperature, impairing protein function, membrane and cell envelope integrity and the performance of chemical processes. Bacteria have evolved several systems to counteract transient and persisting increases in temperatures, universally called the heat shock response for short term, transient mechanisms, and heat adaptation for prolonged survival at supraoptimal temperatures. We studied the adaptation of the thermoalkaliphile *Caldalkalibacillus thermarum* to supraoptimal temperature. Cultures of *C. thermarum* incuabted at the optimal growth temperature of 60°C and then exposed to the supraoptimal temperature of 70°C showed a significant increase in the expression of metabolic enzymes, including a 256-fold increase in myo-inositol-1-phosphate synthase (*mccI*) protein levels. This enzyme catalyzes the conversion of glucose-6-phosphate to myo-inositol-1-phosphate and is involved in the synthesis of the thermoprotectant di-myo-inositol-1,3'-phosphate (DIP). The gene encoding myo-inositol-1-phosphate synthase in *C. thermarum* is part of the *mccXIC* operon encoding a putative CDP-alcohol phosphatidyltransferase (*mccC*), another enzyme of the DIP synthesis pathway.
A degenerated CIRCE element overlaps the start codon of *mccX*, and the kinetics of *mccI* induction after exposure to 70°C indicate that this operon is regulated by HrcA, the negative regulator of class I heat shock response in Gram-positive bacteria.
Further, expression of enzymes involved in the degradation of myo-inositol-1-phosphate to D-glyceraldehyde 3-phosphate and acetyl-coA was found to be upregulated at 70°C. At the same time, the activity of phosphofructokinase Pfk, an enzyme of the upper part of glycolysis, showed a 20-fold decrease. This suggests that exposure of *C. thermarum* to supraoptimal temperature leads to a re-routing of glucose-6-phosphate from glycolysis into inositol synthesis and degradation.

SSP008

S-bacillithiolation protects against hypochlorite stress in *Bacillus subtilis* as revealed by transcriptomics and redox proteomics

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Protein *S*-thiolation is a post-translational thiol-modification that controls redox-sensing transcription factors and protects active site Cys residues of essential enzymes against irreversible overoxidation to sulfonic acids. In *Bacillus subtilis* the MarR-type repressor OhrR was shown to sense organic hydroperoxides via formation of mixed disulfides with the redox buffer bacillithiol (Cys-GlcN-Malate, BSH), termed as *S*-bacillithiolation [1]. We have studied changes in the transcriptome and redox proteome caused by the strong oxidant hypochloric acid in *B. subtilis* [2]. The expression profile of NaOCl stress is indicative of disulfide stress as shown by the induction of the thiol- and oxidative stress-specific Spx, CtsR and PerR regulons. Thiol redox proteomics identified only few cytoplasmic proteins with reversible thiol-oxidations in response to NaOCl stress that include GapA and MetE. Shotgun-LC-MS/MS analyses revealed that GapA, Spx and PerR are oxidized to intramolecular disulfides by NaOCl stress. Furthermore, we identified six *S*-bacillithiolated proteins in NaOCl-treated cells, including the OhrR repressor, two methionine synthases MetE and YxjG, the inorganic pyrophosphatase PpaC, the 3-D-phosphoglycerate dehydrogenase SerA and the putative bacilliredoxin YphP. S-bacillithiolation of the OhrR repressor leads to up-regulation of the OhrA peroxiredoxin that confers together with BSH specific protection against NaOCl. *S*-bacillithiolation of MetE, YxjG, PpaC and SerA causes hypochlorite-induced methionine starvation as supported by the induction of the S-box regulon. The mechanism of *S*-glutathionylation of MetE has been described in *Escherichia coli* also leading to enzyme inactivation and methionine auxotrophy. In summary, our studies discover an important role of the BSH redox buffer in protection against hypochloric acid by *S*-bacillithiolation of the redox-sensing regulator OhrR and of key enzymes of the methionine biosynthesis pathway.
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SSP009

How bacteria take care of their kids: Evidence for controlled and fair distribution of PHB granules to daughter cells in *Ralstonia eutropha* H16

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Ralstonia eutropha H16 has become the model organism for studying metabolism of poly(3-hydroxybutyrate) (PHB), an important biodegradable biopolymer that is sustainable produced worldwide in the scale of 10^5 t/a from renewable resources such as sugars [1].R. eutropha cells usually accumulate about a dozen PHB granules during growth at high C/N-ratios. While biochemistry and molecular biology of PHB accumulation and PHB biodegradation have been investigated in great detail during the last two decades only little is known whether and how subcellular localization of PHB granules is controlled by the bacteria. We addressed this question by performing a two-hybrid approach to screen for proteins with the ability to interact with proteins of the PHB granule surface [2,3]. Two novel Pha proteins were identified which control subcellullar localization of PHB granules and ensure almost equal distribution of PHB granules to daughter cells after cell division as revealed by fluorescence microscopy and transmission electron microscopy. A revised model for PHB granule formation will be proposed.
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SSP010

Food Safety: Is there a positive relationship between heat resistance and dehydration stress of infant pathogen *Cronobacter*?

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Cronobacter bacteria, formerly classified as *Enterobacter sakazakii*, have been implicated in several incidents as the cause of meningitis and enterocolitis with high mortality rates in premature infants resulting from

feeding with contaminated powdered infant formula (PIF) [1]. PIF therefore is strictly recommended to be "sakazakii-free" which is defined as the absence of any colony forming unit in 30 samples of 10g of PIF [2]. Recent studies e.g. [3] noticed a cross-resistance of *Cronobacter* strains pointing to common regulation mechanisms for coping with heat and dry resistance which both play a key role in the production of PIF. Investigating these mechanisms, we are going to develop a modified production process in cooperation with the Food Process Engineering section of our institute and the Milchwerke "Mittelelbe" GmbH, an industrial producer of PIF.

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SSP011

Characterization of *Staphylococcus aureus* persister cells upon daptomycin treatment

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Bacterial cultures contain a subpopulation of dormant cells, persisters. These non-growing cells are phenotypic variants of the wild type that are tolerant but not resistant to antibiotics. As persistence is a transient phenotype it is inherently difficult to study the molecular mechanisms associated with this kind of bacterial dormancy.
Growth phase, strain background, and genotype are critical for the formation of *Staphylococcus aureus* persister cells. According to our results, *S. aureus* cells in stationary growth phase are generally less vulnerable by antibiotics than exponential cultures presumably due to elevated persister levels. We previously identified parameters for the selection of *S. aureus* persister cells. Biphasic killing kinetics, highly indicative of persisters, were observed by exponential-phase cells treated with 10- and 100-fold MIC of tobramycin and ciprofloxacin, respectively. Under stationary growth phase cells challenged with 100-fold MIC of daptomycin showed an initial reduction of viable cell counts within the first hour (99.98%) followed by a plateau of surviving cells with a rather slowly decrease in the amount of CFUs. *S. aureus* SA113 stationary-phase persisters selected by daptomycin treatment are currently being analyzed for alterations in transcriptional and metabolic patterns by microarray and ^{13}C isotopologue profiling, and in morphology via transmission electron microscopy.
The new findings may aid in revealing persister genes in *S. aureus* as well as in deciphering physiologic and cellular states of *S. aureus* persisters.

SSP012

The influence of supercritical CO_2 on sulphate reducing and methanogenic enrichment cultures from hydrocarbon reservoirs in Germany

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Large-scale solutions are needed to reduce the emissions of greenhouse gases such as CO_2 or CH_4 in consequence of their global warming potential. Carbon capture and storage (CCS) offers one option for reducing such emissions with the storage of CO_2 within depleted gas and oil fields. Our study is focusing on the direct influence of high CO_2 concentrations on the autochthonous microbial population and environmental parameters at such sites.
The reservoir within the Schneeren-Husum formation was investigated for its chemical properties, activity profile and microbial community composition via T-RFLP, clone libaries and quantitative-PCR (qPCR). Even within one reservoir differences between two well heads were observed in the inducible activity after substrate addition. Also qPCR analysis showed two distinct communities with varying copy numbers of several bacterial and archaeal genes (16S rRNA, *dsrA, mcrA* etc.). Pyrosequencing data gave insights into the reservoir community in a direct comparison of produced well head fluids and deep reservoir samples (down hole sampling).
High CO_2 had a negative effect on methane and sulphide production in experiments conducted with amended original fluids and enrichment cultures. In a second step original fluids (amended with substrate) from the reservoir were incubated for several weeks under near *in situ* temperature (~50°C) with supercritical CO_2 (100 bar). In this experiment the viability of microorganisms together with community changes were investigated using quantitative PCR, DGGE and CARD-F*I*SH. In conclusion this experiment provides information on possible microbiological changes in the reservoir during and after storage of CO_2.

SSP013

Biogenesis of PHB granules in *Ralstonia eutropha* H16 and in mutants with overexpressed or deleted PhaM and PhaP5 proteins

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Poly(3-hydroxybutyrate), PHB, is the most common polyhydroxyalkanoate and is important for many bacterial species as a carbon and energy source during times of starvation. Prior to this work, a two-hybrid approach was applied to screen for uncharacterized proteins with the ability to interact with PHB synthase (PhaC1) and other PHB-related proteins of *R. eutropha*. As a result, two new proteins - PhaM and PhaP5 - were identified that are involved in biosynthesis of PHB. Both proteins showed interactions with other PHB-associated proteins and with each other and colocalized with PHB granules in vivo (as fusion with eYfp).
A ΔphaM mutant accumulated only one or two large PHB granules instead of several medium-sized PHB granules of the wild type, and distribution of granules to daughter cells was disordered. This phenotype was reversible by substitution of phaM in trans. When PhaM was constitutively overexpressed the cells formed many small PHB granules that were associated with the cell pole-facing side of the nucleoid region. Purified PhaM revealed strong but sequence-independent DNA-binding ability in EMSA experiments in vitro.
A ΔphaP5 mutant showed no significant effect on size and localization of accumulated PHB granules. However, when PhaP5 was constitutively overexpressed, cells formed smaller PHB granules than the wild type and the granules were organized in tight bundles always associated to both cell poles. This phenotype is similar to that of a ΔphaP1-4 mutant. In conclusion, PhaM and PhaP5 determine number, surface to volume ratio, subcellular localization and distribution to daughter cells of PHB granules in *R. eutropha* H16. Subcellular localization of PHB granules in *R. eutropha* depends on a concerted expression of at least three PHB-granule-associated proteins, namely PhaM, PhaP5 and PHB synthase PhaC1.

SSP014

Killing of Biothreat agents on metallic copper surfaces

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Currently there is an increasing interest in metallic copper (Cu) surfaces due to their antimicrobial properties. Using surfaces that might diminish surface related contamination is of great interest in order to improve hygiene. Dry Cu surfaces demonstrated that at both laboratory conditions and hospital trials a wide variety of microorganisms get inactivated. The mechanism by which microbes are killed by dry Cu surfaces is still not fully understood. Nonetheless, a microbe faced with Cu surfaces, is rapidly inactivated within minutes by a quick and high copper uptake. Bacterial species able to evade the host immune system are among the most serious lethal microbial challenges to human health. This group of pathogens comprises biothreat species classified by the Center for Disease and Control and Prevention (CDC) as bacterial select agents with the potential to be misused as bioterroristic weapons.
We investigated the killing effectiveness of Cu surfaces against Gram-negative bacteria that cause high morbidity and mortally rates in humans (*Brucella melitensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Francisella tularensis* and *Yersinia pestis*). The pathogens' inactivation kinetics validates efficient and rapid inactivation on dry Cu surfaces. As control surface we used stainless steel that is known not to be antimicrobial. Furthermore we tested the ability of Cu surfaces to affect the membrane-integrity of bacteria after different Cu-exposition times with so called LIVE/DEAD® staining. By this technique we demonstrated that Cu surfaces damage the membranes of Gram-negative bacteria.
These results can be expected to help reinforcing the idea of applying Cu surfaces, for improving hygiene and to aid in the war against nosocomial- and other infections.

SSP015

Unravelling the function of multiple PHB depolymerases in *R. eutropha*

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Poly(3-hydroxybutyrate) (PHB) is a biodegradable thermoplast that is produced by fermentation of the Gram-negative "Knallgasbakterium" *Ralstonia eutropha* in an industrial scale (≈ 50.000t/a). More than a dozen of proteins that are relevant for PHB metabolism have been previously identified. Nevertheless many problems especially in understanding the processes of intracellular PHB degradation remain unsolved. Up to now,

nine putative PHB depolymerases have been postulated to exist in *R. eutropha*. However, except for PHB depolymerase PhaZa1 [1] only little is known about subcellular localization and *in vivo* activity of the respective gene products. In this contribution, fusions of candidate *phaZ* genes with eyfp were generated and conjugatively transfered to *R. eutropha* HF39. Localization of expressed fusion proteins was determined under condition permissive for PHB accumulation and PHB mobilization. Colocalization of PhaZa1-eYfp with PHB granules was confirmed for PhaZa1 and was also found for PhaZa3, PhaZa4 and PhaZa5 but not for PhaZa2. Fusions with 3HB-oligomer hydrolases (PhaZb, PhaZc) were homogenously distributed in the cytoplasm and a colocalization with PHB granules was never observed. Moreover, PhaZd1, a putative PHB depolymerase with so far highest *in vitro* depolymerase activity with nPHB granules [2], did also not colocalize with PHB *in vivo*. While it is reasonable to assume soluble 3HB-oligomer hydrolases, because 3HB oligomers are water-soluble, the function of soluble PHB depolymerases remains unclear.

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SSP016

Elucidation and studies of a new protein involved in anaerobic phosphite oxidation

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Protein identification is based on the availability of genomic data. Using "bottom-up" proteomics approaches the identification of proteins is often straightforward. In the absence of genomic data it is highly complex or unfeasible and/or typically requires "de novo"- identification approaches.
Here we present the identification approach and some preliminary studies of a new enzyme involved in the anaerobic phosphite oxidation process byDesulfotignum phosphitoxidans(strain FiPS-3), a strictly anaerobic and sulfate-reducing bacterium [1].
In the presense of phosphite as e-donnor we found a specifically expressed protein of a molecular mass around 40 kDa on SDS-PAGE gels. Further proteomic and genetic studies revealed that this is a new protein which we have identified as a putative NAD(P)-dependent epimerase/dehydratase [2], with calculated MW mass of 35.8 kDa. The protein was found in the membrane and in the soluble protein fractions ofD. phosphitoxidans. In addition we have found that 20% of the total phosphite oxidizing activity was in the washed membrane fractions ofD. phosphitoxidans. The estimated molecular weight on 6% native PAGE of this protein is about 140 kDa, which suggests that the protein of interest is a homotetramer. This corresponds to the specific differentially phosphorylated pattern that this protein showed on 2D SDS PAGE. A more detailed functional characterization of the new protein is presently carried out.

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SSP017

Effect of Salt and Matric stress on Growth, Cell Suface Properties, Membrane Composition and Gene Expression of *Pseudomonas putida* mt-2

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Within the framework of the EU-project BACSIN (Bacterial Abiotic Stress and Survival Improvement Network) effects of different environmental stressors on ubiquitously occurring and metabolically versatile microorganisms are investigated in order to enable biotechnological applications for bioremediation or biotransformation. The ubiquitously occurring bacterium *Pseudomonas putida* fulfills these requirements. Therefore, adaptive mechanisms of *P. putida* mt-2 to salt (sodium chloride) and matric stress [polyethylene glycol 6000 (PEG_{6000})] were investigated on the physiological and transcriptional level. Changes in the physiology of the cell were recorded by the analysis of growth, cell envelope hydrophobicity (contact angle measurements) and the charge of the cell envelope (zeta potential measurements). Global transcriptional changes were monitored via DNA-microarrays. The experiments lead to the following results:
(i) Salt-stressed *P. putida* mt-2 grew at lower water activities compared to matric-stressed cells. This suggests that adaptive strategies are more effective during exposition to high salt concentrations. (ii) NaCl had an effect on the fatty acid composition, the hydrophobicity and the surface charge of the cell envelope whereas the matric stressor PEG_{6000} had no influence. With increasing salt concentrations the cell envelope became more hydrophobic, more charged and more rigid. (iii) With the help of the DNA-microarray technology general insights in the household of the cell were obtained. The metabolic activity was restructured due to the influence of the stressor. Generally, several enzymes of the citric acid cycle, the arginine fermentation, the lipid and the pentose phosphate pathway were down regulated, whereas enzymes of the lactic acid fermentation (*lctP*,*lldD*) and aerobic compound degrading enzymes were up regulated. (iv) Finally, taurine or a similar aliphatic sulphate was identified as a possible compatible solute based on the up-regulation of aliphatic sulphate transport systems.

SSP018

Bacterial Interaction Leading to Pattern Formation

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Bacterial communities represent complex and dynamic ecological systems. Different environmental conditions as well as bacterial interactions have determining influence on establishment and conservation of bacterial diversity and can lead to so-called pattern formation. Stable coexistence of several bacterial strains is often only possible under well-defined conditions.
To study the development of bacterial populations we use time-lapse microscopy to investigate the colicin E2 system of threeEscherichia colistrains labeled with different fluorescent proteins. Combinations of these strains, with distinct growth parameters, lead to either instable, metastable or stable coexistence. Besides growth rate and colicin production, coexistence was mainly influenced by lag time variations. In accordance with the results, two main strategies lead to survival: sensitive strains need short lag phases and rapid growth rates, while toxin producing strains even with extended lag phases and slower growth rates can prevail. Specific growth parameters enable cyclic dominance, where the colicin-producing strain kills the sensitive strain, outgrows the resistant one. This in turn has a growth advantage over the first.

SSP019

RecA-mediated LambdaSo prophage induction in *Shewanella oneidensis* MR-1 biofilms

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The respiratory versatile γ-proteobacterium *Shewanella oneidensis* MR-1 has emerged as a model system for biofilm formation of environmental bacteria. Our laboratory recently demonstrated that extracellular DNA is an important structural component in all stages of biofilm formation, and that deletion of prophages (LambdaSo, MuSo1, MuSo2) correlates with a significant reduction in cell lysis and eDNA release. In order to characterize LambdaSo prophage induction in *S. oneidensis* MR-1 biofilms in time and space, we generated MR-1 strains carrying *venus* as transcriptional fusion to regulatory and assembly genes in the LambdaSo prophage genome. Biofilm development under hydrodynamic conditions and prophage induction was monitored by confocal laser scanning microscopy. Our results strongly indicate that induction of prophage LambdaSo occurs 24 hours after initial attachment. Interestingly, significant fluorescence correlated with a filamentous morphology of cells that were evenly distributed in the biofilm, but absent in microcolonies. Similar filamentous structures that were mutually exclusive to cells exhibiting Venus fluorescence were also visible after staining eDNA, suggesting induction of cell lysis after filamentation. Since activation of the RecA-mediated SOS-response in *E. coli* induces filamentation and Lambda prophage induction, we determined whether *recA* is responsible for LambdaSo induction in *S. oneidensis* MR-1 biofilms. Deletion of *recA* completely abolished *venus* expression during all stages of biofilm development, indicating suppression of LambdaSo induction. Addition of hydrogen peroxide to planktonic cultures strongly increased both filamentation and prophage induction, and moreover, considerable hydrogen peroxide levels were detected in biofilm associated cells. Based on these results, we hypothesize that LambdaSo induction is under control of RecA under biofilm conditions and that oxidative stress may be a direct stimulus.

SSP020

Hot trehalose: A report about the unusual bifunctional TPSP pathway in *Thermoproteus tenax*

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The widespread non-reducing disaccharide trehalose, consisting of two a-1,1 linked glycosyl-glucose molecules, is known to function as compatible solute in Eucarya and Bacteria, protecting the cell against a wide range of different stress conditions [1]. Trehalose has been identified in Archaea, but its function is still unknown.
The (OtsA/OtsB) TPS/TPP pathway via trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP) is the most common pathway for trehalose synthesis. UDP (ADP-) glucose and glucose-6-phosphate are transformed into trehalose-6-phosphate by TPS and subsequently dephosphorylated by TPP forming trehalose and P_i [2]. In the genome of the hyperthermophilic Crenarchaeon *Thermoproteus tenax* an operon comprising a gene coding for a trehalose-6-phosphate synthase/phosphatase (*tpsp*), with a C-terminal TPS- and N-terminal TPP-domain was identified [3]. This operon also harbors a putative glycosyl transferase (*gt*) and a putative small conductive mechanosensitve channel (*msc*). The two-domain TPSP structure has already been described for plants (e.g. *Selaginella leptophylla*, *Arabidopsis thaliana*) and for *Saccharomyces cerevisiae*, but these TPSPs only possesses one activity, either TPS or TPP. Only recently a bifunctional TPSP activity has been reported from *Cytophaga hutchinsonii* [4]. For the Archaeon *T. tenax*, biochemical studies of the recombinant protein revealed a TPSP with full TPP activity and only in the presence of GT bifunctional TPSP activity was observed. In our current model, we suggest that GT activates TPSP by complex formation. The MCS might function as the emergency valve which allows the maintenance of the cell turgor in order to respond to environmental cues (e.g. osmotic stress).

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SSP021

Evolutionary stabilisation of bacterial cooperation by switches between microcolonial and planktonic life style

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Mechanisms ensuring evolutionary stability of bacterial cooperation are not well understood. Bacterial autoinducer (AI) signals, i.e., diffusible molecules released by bacterial cells, enable a cooperative and coordinated gene expression on population level [1]. This behaviour has been described as quorum sensing or efficiency sensing. Cheater mutants save costs by not producing the signal molecule or by avoiding the AI regulated cooperative phenotype expression. Therefore, they should outcompete cooperative cells. It has been proposed that kin selection mechanisms within microcolonies, grown from common ancestors and thus composed of closely related cells, may promote the stability of AI systems. As AI systems have also been described in plankton, and many bacteria species switch between colonial and planktonic life style, a natural question arises: Can life style changes evolutionary stabilize AI functionality in plankton? As a first approach, we analyze this theoretically within a mathematical model. We assume costly AI production, an AI regulated costly phenotype expression (as e.g. exoenzyme production), phenotype dependent logistic growth of the colonies and plankton, stochastic changes between microcolonial and planktonic life styles, cell as well as colony death, and mutation from wildtype to AI resp. exoenzyme deficient mutants. First results indicate that life style switches can have stabilizing effects that support an equilibrium between wildtype and cheater cells. The fraction of cheater in the stationary state depends, among others, on the exchange rate between microcolonies and plankton as well as the colony death rate.

[1] Hense et al. (2007) Does efficiency sensing unify diffusion and quorum sensing? Nat. Rev. Microbiol. 5: 230-239

SSP022

Fatty acid-independent adaptation of bacterial membranes to cold temperatures

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The adaptation of microorganisms to low temperatures is one of the most important adaptations to extreme conditions because cold environments represent the majority of the biosphere on earth. Furthermore, psychrotolerant and psychrophilic microorganisms are of special interest in the food industry with respect to food protection, safety and quality.
One of the most significant adaptations of microorganisms to cold temperatures is the control of cell membrane fluidity. Membrane fluidity is usually controlled by adaptations of the fatty acid profiles. However, during the last years we analyzed psychrotolerant bacterial isolates from different sources, Arctic and Antarctic soil samples, chilled food samples and refrigerators, which show no or unexpected adaptations of their fatty acid profiles when grown under low temperatures. For these isolates we expect other mechanisms involved in the modulation of membrane fluidity.
In this study we focused on the alteration of the cell quinone content as mechanism of membrane adaptation. We hypothesize that increase of quinone concentration may result in an increase of the disorder of membrane fatty acid acyl chains disorder analogue to other lipophilic membrane fluidizers. Therefore, quinones may be an alternative to fatty acid related effects like increase of monounsaturated fatty acids. We analysed several psychrotolerant bacterial strains for changes of their fatty acid profiles and quinone content when grown under different temperatures. For most strains we found a decrease in the quinone content under low temperature conditions. However, some strains of the *Bacillaceae* showed increase of their quinone content under low temperature growth conditions in accord with our working hypothesis. From there, we hypothesize that for some bacterial taxa quinones play a role in the adaptation to cold temperatures and in the control of cell membrane fluidity.

SSP023

Essential oils show specific inhibiting effects on biofilm formation by bacteria

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The use of essential oils as food preservatives gets more and more interesting in the food processing industry. We analysed the inhibiting effects of thyme, oregano and cinnamon essential oil on biofilm formation by strains of the genus *Acinetobacter*, *Sphingomonas* and *Stenotrophomonas*. These biofilm forming test strains were isolated from authentic biofilms in the food industry during a previous study.
Minimal inhibitory concentrations (MIC´s) for growth and biofilm forming activity were tested in a 96-well microtiter plate assay. Biofilm forming activity was tested based on a crystal violet assay. For some strains inhibition of growth and inhibition of the biofilm formation by the essential oils are initiated at the same concentration. However, for strains of the genus *Acinetobacter* and *Sphingomonas* we found an inhibiting effect of essential oils on biofilm formation considerably below the MIC for growth of these strains. Thyme oil is capable to inhibit the development of a biofilm at low concentrations up to 0,002 %. This natural substance seems to be the most efficient specific inhibitor compared with the other tested essential oils against the biofilm formation of all tested isolates. Controls showed that the detergent used, Tween 20, was not responsible for this effect. The same tests were carried out with the main components of the essential oils. Fluorescence microscopy was performed to visualize the structural change of the biofilm after application of sublethal concentrations of essential oils.
The effective concentration of the natural substances was dependent on the type of essential oil. The strains showed different sensitivity depending on the oil.

SSP024

Thermal stabilization of procaryotic ribosomes by compatible solutes

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Ribosomes play an important role in cell metabolism. Besides integrity of the cell membrane, ribosomal function is supposed to determine the temperature limit of life [Gaucher 2008, Lee 2002]. Ribosome stability under physical stress has been investigated for some time but so far the influence of the solvent water and its modulation by co-solvents has been ignored.
In this context the well-known stabilizing effect of compatible solutes (osmolytes) on proteins is subjected to scrutiny because ribosomes also comprise base-paired nucleic acids, for which a destabilizing effect had to be expected (Lambert 2007).
Here we present a first insight into the effects of some protein-stabilizing and -*de*stabilizing low molecular weight osmolytes on *E. coli* and *H. elongata* ribosomes under thermal stress. Ribosomal stability in the presence and absence of co-solutes was investigated using differential scanning calorimetry according to the methods of Lee [2002] and Mackey

[1991]. We were able to demonstrate that solute-induced protein stabilization does not always correlate with ribosome stabilization. Furthermore, depending on the solute, we observed different effects in relation to the subunits (30S and 50S) as compared to the fully functional 70S ribosome.

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SSP025

Elucidation of potential vitrificants of *Halomonas elongata* DSM 2581^T with regard to desiccation tolerance and bio-inspired use as interface protectants

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The loss of water is a general stress phenomenon for life on earth. However, some extremophilic organisms are able to survive almost complete desiccation by vitrification, a process termed anhydrobiosis or cryptobiosis. Under these conditions the whole metabolism is arrested and the cells can remain dormant for a long period of time until they are rehydrated. This survival mechanism preserves macromolecules (e.g. proteins, DNA, membranes) by the formation of biological glasses at the nano-structured interface to the environment. Well-known glass-forming substances (sugars and other hydroxyl-group carrying compounds) are believed to replace water molecules in the hydration shell of biological boundaries and thus prevent complete inactivation by the lack of water. The present work aimed at the identification of such glass-forming compounds in *Halomonas elongata* and other factors involved in vitrification. Molecular candidates are hydroxylated derivatives of ectoine (S,S-beta-hydroxectoine), highly hydrophilic and intrinsically unstructured proteins (so-called hydrophilins), but also inorganic ions and salts of organic acids [1-4]. As an experimental approach to elucidate the organism´s response to a forthcoming dehydration event the exponentially growing culture was exposed to gradually increasing temperature beyond maximum. In addition, bioinformatic data were exploited to predict potential hydrophilins and to investigate other characteristics of the *Halomonas elongata* proteome.

It was shown that, besides the expected accumulation of hydroxylated forms of compatible solutes, *H. elongata* is also able to express so-called hydrophilins to support vitrification. In addition, its moderately acidic proteome may provide an additional basis for increased water stress tolerance.

Detailed understanding of all factors involved in vitrification and preservation of biological surfaces which depend on water for function and integrity will ultimately enable us to apply such knowledge for the long-term stabilization of immobilised enzymes and biohybrid interfaces as for example in technical biosensors.

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SSP026

The CRISPR/Cas system of Haloferax volcanii: requirements for the defence

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The CRISPR/Cas system is a prokaryotic defence system that provides adaptive and heritable immunity against foreign genetic elements in most Archaea and many Bacteria. This system is widespread and diverse, but as it was only recently discovered, the precise molecular details for the defence directed against invading plasmids or viruses are far from understood. Clustered regularly interspaced short palindromic repeats (CRISPRs) together with CRISPR associated genes (casgenes) build the basis of the system. The so-called spacer sequences in a CRISPR locus are derived from the invading nucleic acids (protospacer) in the adaptation stage, to enable recognition and degradation in case of re-infection (interference stage). Furthermore, specific short sequences called PAMs (protospacer adjacent motifs) are essential for the adaptation and interference of most CRISPR/Cas types. We investigate the mechanisms of the CRISPR/Cas-mediated defence in the Euryarchaeon Haloferax volcanii- an organism for which the origin of spacer sequences remains totally elusive and thus the role and identity of PAM sequences was unknown until now. Using a plasmid assay for which a protospacer identical to the first spacer of oneH. volcaniiCRISPR locus is combined with all potential 2 nt- or 3 nt-PAM sequences, we identified two PAM sequences so far. We further investigate, to which extent sequence identity between spacer and protospacer must be given to ensure a successful interference reaction.

SSP027

Succession patterns of distinct flavobacterial groups after spring algal blooms in the North Sea

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Algae blooms are known to cause significant changes in bacterioplankton composition. During and after the algal spring bloom in 2009 a large microbial community shift was observed in the North Sea. 16S rRNA tag sequencing at different timepoints revealed that *Alphaproteobacteria* and *Gammaproteobacteria* as well as the *Bacteroidetes*, here in particular the class *Flavobacteria*, dominate the bacterioplankton community in the North Sea at Helgoland Roads. In this study we dissected the flavobacterial response on algal blooms. Specific oligonucleotide probes for *Flavobacteria* clades were designed, and these clades were quantified by catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) and automated microscopy.

In spring 2009 a tight succession of distinct *Flavobacteria* clades was observed. Members of the genera *Ulvibacter* and *Formosa* reached relative abundances of up to 20% and 24%, respectively, within one to two weeks after the peak of the algal bloom. These groups seem to respond to specific substrates released by the algae after the bloom (bottom-up effect). Later, while *Ulvibacter* and *Formosa* subgroups dropped the *Polaribacter* clade increased up to 27% of the entire microbial community. Interestingly, all analysed subgroups were present throughout the rest of the year 2009 only in low abundances, except for the *Polaribacter* clade which showed several peaks during the course of the year in response to the summer and autumn algal blooms. All flavobacterial subgroups responded more strongly to the diatom-dominated spring bloom than to the autumn bloom composed mainly of green algae.

In 2010 the spring phytoplankton bloom occurred one month later than in 2009, but a similar succession of the flavobacterial groups could be observed reaching similar cell numbers. Our findings suggest that the wax and wane of specific bacterioplankton clades might be an annually recurring phenomenon in the North Sea, and therefore rather a deterministic than stochastic process.

SSP028

Host- and cell type-specific adhesion of human and animal *Escherichia coli* in association to their virulence-associated genes

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Escherichia coli (E. coli) is a common bacterium of the intestinal microflora of mammals and birds but also can cause intestinal as well as extraintestinal disease. Pathogenic *E. coli* can be grouped into several pathovars including host-specific and zoonotic bacteria.

Successful colonization and infection of epithelial cells depend on initial adhesion which is mediated by fimbriae and other adhesins or colonization factors. Adhesins promote host- and tissue specificity or enable bacteria to colonize a broader range of hosts and tissues which are two different survival strategies.

We sampled 410 pathogenic and commensal *E. coli* isolates from humans and 19 mammalian and avian species including domestic and wild animals and including isolates from faeces and the urinary tract.

All strains were tested for hemolysis on blood agar plates and for 42 virulence-associated genes (VAGs) including several adhesins with a VideoScan Multiplex-PCR-Bead-Assay which was developed in our laboratory. All non-hemolytic isolates (n= 296) were analyzed for adhesion to four epithelial cell lines (Caco2: human intestinal, 5637: human urinary bladder, IPEC-J2: porcine intestinal, PK15: porcine kidney) automatically with our new developed VideoScan technology. Adhesion pattern were correlated with the presence of VAGs and VAG pattern.

In average, hemolytic isolates carried twice as many VAGs compared to non-hemolytic isolates. Isolates had a species-specific repertoire of VAGs. Adhesion pattern strongly varied between isolates independent from species origin. Adhesion of bacteria could be divided into non-adherent,

cell line-un-specific adherent and cell line-specific adherent. We defined VAGs (e.g.sfa/foc,malX) which presence was associated with higher adhesion to one specific cell line and thus host and/or tissue specificity. However, there were no differences in adhesion rates between pathogenic and commensal isolates.
In conclusion, we show a broad variety of VAG and adhesion pattern in human and animal *E. coli* isolates. Adhesion is host- and cell type-specific enabling colonization of different microhabitats. There are confirmed adhesins but other hypothetical and yet unknown adhesins and their host and tissue specificity have to be identified and characterized in future studies.

SSP029

A role for glutamine synthetase in regulation of proline biosynthesis

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Glutamine and glutamate are the major compatible solutes in the moderate halophile *Halobacillus halophilus* under moderate salinities and proline at high salinities (1). One of the isogenes/-enzymes of glutamine synthetase (*glnA2*) was shown before to be the osmoregulated key player in salinity-dependent glutamine and glutamate biosynthesis (2). Here, we have deleted *glnA2* from the chromosome of *H. halophilus*. Growth of the mutant was not impaired, neither at low nor at high salt, and the mutant did not have reduced levels of glutamine or glutamate, indicating a metabolic bypass for glutamine and gutamate production. Much to our surprise, the mutant did no longer produce proline as compatible solute. The loss of proline was compensated for by increased ectoine production. Consistent with this is the observation that the transcript levels for *ectA* (and *glnA1*) were increased in the mutant.
Our data demonstrate a regulatory role of glutamine synthetase 2 in proline biosynthesis. Possible regulatory scenarios are discussed.
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SSP030

The impact of a typical biofilm flora on the VBNC-state of pathogens in drinking water biofilms

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Pathogens like *Legionella pneumophila*and *Pseudomonas aeruginosa* are known for their ability to persist in house installations and pose a risk of infections for humans. The standard approach for detection is still the heterotrophic plate count. But it is also common, that over 90 % of the microorganisms in such oligothrophic environment are not growing on known media.
Only a few studies focus on the typical biofilm flora in drinking water, which is shown to be a mixture of mainly α- , β- and γ-subclasses of Proteobacteria. These bacteria can turn into a physiological state called "viable-but-not-culturable" (VBNC), in which no growth can be observed on plates. The bacteria show dwarfish cell forms and reduced metabolic activity.
In our investigations we study how these bacteria have an impact on the entrance or exit of *P. aeruginosa* in the VBNC-state. Therefore a biofilm reactor was developed to simulate a low nutrient environment and to induce VBNC in *P. aeruginosa* as well as other selected bacteria, which were isolated in our group from native biofilms.
Mono-species biofilms and multi-species biofilms with integrated pathogens are compared by using culture- (e.g. CFU) and culture-independent methods like Live/Dead staining, PAC (a direct- viable-count method), qPCR, FISH and CLSM. The aim of this project is to gain insights in which manner *P. aeruginosa* can persist in drinking water biofilms and how native bacteria influences the resistance of *P. aeruginosa* in water plumbing. Furthermore the reproducible induction of VBNC by low nutrients (carbon, phosphate or trace elements) are being considered and investigated.

SSP031

The CRISPR-Cas system of *Haloferax volcanii*

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The recently discovered CRISPR-Cas system (CRISPR:clustered regularly interspaced short palindromic repeats, Cas: CRISPR-associated) is an adaptive and heritable resistance mechanism against foreign genetic elements. The CRISPR-Cas system consists of clusters of repetitive chromosomal DNA in which short palindromic DNA repeats are separated by short spacers, the latter being sequences derived from the invader. In addition, a set of proteins, the Cas proteins, is involved. We are investigating the CRISPR-Cas system in the halophilic archaeon *Haloferax volcanii*. *H. volcanii* is an archaeal model organism which requires about 2.1 M NaCl for optimal growth and raises the intracellular salt concentration to similar values to cope with high salt concentration in the medium. The genome is sequenced and *Haloferax* is one of the few archaeal organisms where genetic systems are available.
H. volcanii has three CRISPR loci, one located on the chromosome and two located on one of the chromosomal plasmids. Next to one of the CRISPR loci the Cas proteins are encoded in one long multicistronic operon including genes for Cas1-8.
We are analysing the expression and processing of the CRISPR RNA. To that end we generated a deletion strain for the *cas6* gene and investigated its effect on CRISPR RNA processing.

SSP032

Copper impacts the gold toxicity in *Cupriavidus metallidurans*

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Cupriavidus metallidurans could be responsible for the formation of bacteriogenic secondary gold nanoparticles [1,2]. We investigated if gene clusters that are up-regulated after treatment with gold complexes might be involved in this process. The megaplasmids of strain CH34, pMOL28 with a chromate and pMOL30 with an extensive copper resistance determinant, are not required for the formation of colloidal particles visible in TEM, which might be gold nanoparticles. A hypothesis that considered a gold transformation process by the *gig* (gold induces genes) cluster products reminiscent to mercury transformation by *mer* gene products could not be verified. Cells pre-incubated with non toxic concentrations of copper displayed increased copper resistance as expected, however, in some *C. metallidurans* strains, gold resistance decreased in parallel to increasing copper resistance in the copper-treated cells. This demonstrated that handling of gold ions by some - but not all - proteins involved in copper resistance led to enhanced toxicity of gold. Deletion of other genes involved in copper resistance led to a simultaneous decrease of copper and gold resistance but not in all strains. All these data demonstrated that copper resistance systems in *C. metallidurans* are involved in transformation of gold, however, not always to the advantage of the cells. The intriguing network of the copper and gold-handling factors in *C. metallidurans* is thus very complicated and needs a detailed in-depth analysis.
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SSP033

A combined transcriptomic and proteomic investigation into the osmoregulatory mechanisms of *Halomonas elongata* DSM 2581^T

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The halophilic γ-proteobacterium *Halomonas elongata* thrives at a wide range of salt concentrations by accumulating the compatible solute ectoine. Ectoine can be amassed in the cytoplasm either by synthesis or by transport from the medium. To enable ectoine uptake, *H. elongata* is equipped with a specific transport system, named TeaABC. TeaABC is not only required for the accumulation of ectoine, but also functions as a salvage system for ectoine leaking out of the cell. This observation led to the hypothesis that TeaABC and potential efflux protein(s) might be involved in regulating the cytoplasmic ectoine concentration. In order to identify membrane proteins involved in the efflux of compatible solutes and to gain more information about the changes in the protein composition of halophiles in response to salt stress, we analyzed i) the transcriptome and ii) the membrane-proteome of *H. elongata*. By applying a ^{15}N isotope metabolic labeling strategy, a total of 135 membrane proteins were identified and quantified which are significantly up or down regulated in response to changes of the external salinity. In cells adapted to low salt medium (100 mM NaCl) the level of 90 proteins has changed significantly compared to cells adapted to the optimal salt concentration of 1 M NaCl. The content of only 45 proteins was changed when cells were adapted to high salt medium of 2 M NaCl compared to cells grown at 1 M NaCl. The majority of the 135 regulated proteins are putative transport proteins. Four

of these proteins are putative mechano-sensitive channels, of which two were mutated and further analyzed concerning their role in ectoine efflux.

SSP034

The ZIP (ZRT/IRT protein family) member ZupT from *Cupriavidus metallidurans* CH34 has pleiotropic effects on zinc homeostasis

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The well-studied heavy-metal resistant bacteria *Cupriavidus metallidurans* harbors a network of metal efflux systems, which allow survival in heavy-metal polluted environments. These efflux systems remove in a "worry later" scenario surplus cytoplasmic metal cations that were previously imported into the cell by a variety of highly redundant metal uptake systems. To understand the contribution of these metal uptake systems to metal resistance, a systematic deletion analysis of the genes *zupT*, *pitA*, $corA_1$, $corA_2$, $corA_3$, *zntB*, *hoxN*, *mgtA* and *mgtB* was performed. Expression of the genes for all of these transporters was down-regulated by increasing zinc concentration while that of zupT was up-regulated by zinc starvation. ZupT was required for import of zinc at conditions of zinc starvation. The Δ*zupT* deletion strain produced the largest and zinc-containing subunit of the DNA-dependent RNA polymerase, RpoC (beta prime) in excess, and accumulated this protein in inclusion bodies, indicating disturbance of zinc homeostasis, although growth of the mutant strain was not impaired. Additionally, plasmid-bound expression of the *czcCBA* genes encoding the Czc transenvelope efflux protein complex led to disappearance of CzcA in various Δ*zupT*-containing mutant strains. This all indicated a central role of ZupT in zinc homeostasis.

SSP035

BapA is required for biofilm formation in poor-phosphate medium and modifies the structure of the biofilm produced by *Salmonella enterica* sv. Typhimurium

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Salmonella Pathogenicity Island 9 (SPI9) encodes a type 1 secretion system (T1SSs) and its substrate BapA. BapA was associated with biofilm formation but its role during host infection remains unknown. In order to find out the expression conditions and its role in biofilm formation, luciferase reporter and mutant strains of the *bap*-operon as well as *csgDBA* mutant strains were created by lambda-red recombination using as background the NCTC 12023 (WT) strain. Western Blot (WB) and immunofluorescence (IF) were performed using a rabbit antibody anti-BapA, kindly provided by Dr. Lasa, Spain. Strains were incubated either with rigorous shaking or in static conditions for 72 h at 37° or 30° C. As phosphate concentration was described to induce the biofilm formation, PCN with 0.4, 1 and 25 mM PO_4^{-3} was used in addition to LB. Biofilm formation was evaluated with crystal violet in 96 well plates. Bacterial pellets and their supernatant were taken from 2 to 72 h at several time intervals for WB and expression kinetics. Luciferase activity demonstrated that in static conditions *bapA* and *bapD* was around 10-fold higher at 30° C than at 37° C in static conditions after 8 h in WT background. *bapA*-deficient strain was produced 4.0-fold less biofilm, as evaluated by crystal violet, in PCN 0.4 or 1.0 mM PO_4^{-3} than in LB or PCN 25mM PO_4^{-3} in which the mutation did not have any effect. Biofilm formation on glass slides revealed several cluster patterns.*bapA*-decifient strains formed columnar clusters after 96 h which were 10-fold larger than those formed at the same time by the WT strain. Secretion of BapA in WT strain was observed after 120h of static incubation in LB. BapA-positive bacterial cells showed a decreased signal of GFP, which was used as marker for bacterial cells, in contrast to those bacterial cells without BapA. BapA-psoitive cells were also featured by forming isolated groups of bacteria consisting of approx. 10 cells. These results showed that BapA is required for biofilm formation under restrictive low phosphate conditions and that the secretion of BapA is associated with the architecture of the biofilm. Current work is on progress to understand how BapA can modify the architecture of the biofilm and what regulation mechanism controls the expression and secretion of BapA.

SSP036

Molecular approaches to determine the diversity of human adenoviruses present in sewage-contaminated water

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Human adenoviruses (hAdVs) are promising candidates for monitoring viral health risk from environmental water sites. Relatively harmless but common these DNA viruses persist continuously within the population and are routinely detected in polluted surface and wastewater. The 58 currently known serotypes, classified into seven subgenera (subgenus A to G) on the basis of biochemical and biophysical properties, cause a wide range of infections with manifold clinical manifestations such as gastroenteritis, conjunctivitis and respiratory diseases. Different serotypes were reported with different frequencies, showing a prevalence of enteric adenoviruses in water samples, though other serotypes were occasionally detected. The UBA stream and pond simulation system (FSA) allows for tracking of viruses in sewage-contaminated surface water under defined environmental conditions. Adenoviruses do not display strong seasonal fluctuations, but the prevalence of certain serotypes in sewage may change over time, both for epidemiological and virus stability reasons. Therefore the diversity patterns of human adenoviruses from sewage contaminated water were investigated during four long time-experiments carried out in the FSA from 2009 to 2010 using different molecular biological methods, including denaturing gradient gel electropheresis (DGGE) adapted for the detection of human adenoviruses by our group. Additionally, representatives from every subgenus were characterized regarding their stability within the water used. According to our results, human adenovirus serotype 41 was the most prominent adenovirus detected in the samples. Since quantification was connected to PCR amplification the melting points of adenoviral qRT-PCR products were also determined, promising yet another method for rapid diversity investigations. The results indicate applicability of the approaches for other virus groups, including human norovirus genotype analysis from sewage samples and may support the search for viral indicators.

SSP037

Missing protection of DNA by Dps does not enhance toxicity of metallic copper in *Escherichia coli*

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Escherichia coli protects itself from toxic copper ions by several systems. The cytoplasmic membrane localized, coppertransporting P-type ATPase CopA extrude Cu(I) out of the cytoplasm (1). The copper efflux system CusCFBA as well as the multicopper oxidase CueO detoxify the periplamic space from excess copper. The cytoplasmic factor Dps which is abundant in the stationary phase, seems to be involved in copper homeostasis too (2). Dps (DNA binding protein of starved cells) is able to bind the DNA to protect it from oxidative damage (3). Recent studies of survival of *E. coli* on metallic copper leads to the assumption that killing of the bacteria is proceeds by membrane damage, cell death and DNA damage (4). Another study contrary concluded the DNA as the main target of copper toxicity followed by rapid DNA degradation and cell death (5).
The role of Dps in copper toxicity mechanisms in *E. coli* was determined by growth experiments under the influence of ionic copper as well as on metallic copper surfaces.

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SSP038

Cloning, expression and purification of extracellular serine protease Esp, a biofilm-degrading enzyme, from *Staphylococcus epidermidis*

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Staphylococcus epidermidis Esp, an extracellular serine protease, inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. To further expand the biotechnological applications of Esp, we developed a highly efficient and economic method for the purification of recombinant Esp based on a *Brevibacillus choshinensis* expression-secretion system. Theespgene was fused with the N-terminal Sec-dependent signal sequence of the *B. choshinensis* cell wall protein and a C-terminal hexa-histidine-tag gene. The recombinant Esp was expressed and secreted into the optimized medium as an immature form and subsequently activated by thermolysin. The mature Esp was easily purified by a single purification step using nickel affinity chromatography and showed proteolytic activity as well as *S. aureus* biofilm destruction activity. The purification yield of the developed extracellular production system was 5 mg recombinant mature Esp per 20-ml culture, which was much higher than that of an intracellular production system in *Escherichia coli* (3 mg recombinant Esp per 1-l culture). Our findings will be a powerful tool for the production and purification of recombinant Esp and also applicable to a large variety of recombinant proteins used for basic researches and biotechnological applications.

SYV1-FG

The road ahead for microbial systematics: raising our game in the post-genomic era

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Microbial systematics and taxonomy are vital in providing a sound ramework for the activities of all microbiologists. Understanding microbial diversity can be considered one of the key goals of systematics and this activity has been revolutionised by molecular sequence-based approaches. Although formal descriptions of novel taxa rightly remain a cornerstone of taxonomy, it is a concern that most 'traditional' practice now consists of simply describing novel taxa (typically at the genus/species levels). As the vast majority (>75%) of novel descriptions are based on single strains, a pragmatic assessment of current practice indicates that many of the phenotypic tests performed are of questionable taxonomic value and necessity. In this context, it is therefore disappointing that there are relatively few synoptic studies performed that re-evaluate or extend established taxonomies, including placing the taxonomy in an ecological context. Moreover, the principles and practice of prokaryotic systematics have not yet successfully accommodated the dramatic impact of the availability of rapidly increasing numbers of whole genome sequences. This technological shift suggests that a significant reappraisal of the procedures used to describe novel prokaryotic taxa is needed, including defining new minimal standards and the likely introduction of new publication formats. Action is urgently needed if an authoritative framework (including the type system) is to be maintained and in order to sustain systematics as an attractive career choice for 21st century microbiologists.

SYV2-FG

The purpose of prokaryote systematics; clarifying muddy waters

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It is now generally accepted that prokaryotes (members of the Bacteria and Archaea) constitute one of the most diverse and on an evolutionary scale the oldest groups of organisms on this planet. At the same time "microbiology" is one of the younger of the biological sciences. The combination of existing technologies (and the associated knowledge) together with the development of new methodologies presents the microbiologist with the opportunity to gather new information on prokaryotes, to re-evaluate existing data as well as to bridge the gap between existing information and that which is being gathered from newer methods. While systematics has a clear role to play this can only take place if one appreciates the scope of this branch of the natural sciences. The purpose of this talk is to fathom the depths and chart the waters of prokaryote systematics.

SYV3-FG

A single-cell sequencing approach to the classification of large, vacuolated sulfur bacteria

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The colorless, large sulfur bacteria have an intriguing appearance as they can be enormous in size and extremely abundant in sulfidic habitats[1-3]. They were first discovered in 1803[4] and have hence been classified according to their conspicuous morphology. However, morphological criteria have frequently proven to be misleading for the prediction of phylogenetic relatedness in microbiology. Sequencing of several 16S rRNA genes of large sulfur bacteria indicated inconsistencies between the morphologically determined taxonomy and the genetically derived classification, leading to polyphyletic taxa [5-6]. However, a major obstacle to properly reclassify this group is the general failure in growing most of them in pure culture. In the present study, we sequenced nearly full-length 16S rRNA genes and the internal transcribed spacer (ITS) regions from individual hand-picked single cells and filaments of large sulfur bacteria. For each individual, the specific morphology was recorded as well. We found that morphology was strongly misleading in this group of bacteria as on the one hand several morphologies clustered within one species and, on the other hand, some morphological features clustered into several species, genera or even families.

In this study, we included the yet partially sequenced members Thiomargarita namibiensis, Thioploca araucaeand Thioploca chileae, and sequenced also newly identified types of large sulfur bacteria. Based on 128 nearly full-length 16S rRNA-ITS sequences and intending a prospective reclassification we propose nine novel Candidatusspecies along with seven Candidatusgenera. We furthermore suggest the retention of the family Beggiatoaceae, as opposed to Thiotrichaceae[7].

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SYV4-FG

A phylogeny-compliant revision of the systematics for the basal fungal lineages: Chytridiomycota and Zygomycota

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he goal of modern taxonomy is to understand the relationships of living organisms in terms of evolutionary descent. Thereby, the relationships between living organisms are understood in terms of nested clades - every time a speciation event takes place, two new clades are produced. Life comprises three domains of living organisms, these are theBacteria, theArchaeaand theEukaryota. Within the eukaryotic domain the fungi form a monophyletic group of the eukaryotic crown group, and are thus high up in the evolutionary hierarchy of life. Fungus-like organisms possess certain morphological features of fungi, such as the hyphal organization of theOomycotaor the spores and reproductive structures inside a fructification of plasmodiophorids (Plasmodiophoromycota) and slime moulds (Mycetozoa). TheFungisensu strictocomprise a heterogenous group of microorganisms which (i) are primarily heterotrophic with an (ii) osmotrophic style of nutrition containing (iii) chitin and its derivatives representing key compounds in a rigid cell wall during major stages of their life cycle. The most basal fungal lineages are the zoosporic chytrids and the zygosporic fungi forming the transition in evolution of aquatic and terrestrial life style. The systematics of basal fungi is subject to scientific debate. Here, a novel concept, which resolves the systematics in a phylogeny-compliant manner [1-4], will be presented and discussed with respect to concepts of the past [5, 6] and their impact on fungal nomenclature.

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YEV1-FG

New and old tricks in the biogenesis of mitochondrial outer membrane proteins

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The mitochondrial outer membrane contains a diverse set of proteins that mediate numerous interactions between the metabolic and genetic systems of mitochondria and the rest of the eukaryotic cell. All these proteins are nuclear-encoded, synthesized in the cytosol and harbor signals that are essential for their subsequent import into mitochondria. We investigate the molecular mechanisms by which mitochondrial outer membrane proteins are targeted to mitochondria, inserted into the outer membrane and assembled into functional complexes. Evolutionary conserved import pathway for -barrel proteins as well as novel biogenesis processes for helical proteins will be discussed.

YEV2-FG

Mechanistic insight into receptor endocytosis and endosomal A/B toxin trafficking in yeast

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Yeast strains infected with the M28 dsRNA killer virus secrete a heterodimeric killer toxin (K28) belonging to the family of microbial A/B toxins. After receptor-mediated cell entry, the toxin reaches the cytosol of a target cell by travelling the secretion pathway in reverse [1]. The alpha toxin inhibits DNA synthesis in the nucleus and causes apoptotic cell death [1]. Key components in the intoxification process are the β-C-terminal HDEL motiv of the toxin and its interaction with the HDEL receptor Erd2p of the target cell. Most recent CSLM data in conjunction with Erd2p-based reporter assays indicated that Erd2p colocalizes to the plasma membrane where it functions as membrane receptor for toxin endocytosis. Sequence analysis of Erd2p revealed N- and C-terminal endocytic motifs relevant for receptor internalization. Physical Erd2p/Rsp5p interaction identified via BiFC analysis indicated that receptor (mono)ubiquitiation triggers the internalization of receptor-bound toxin. Additional studies verified the importance of early mediators of endocytosis, including the coat building complex and the actin machinery for toxin uptake as well as AP2-complex components, which so far have only been described to be involved in the endocytosis of mammalian cells [2]. To further dissect toxin trafficking, biologically active K28/mCherry fusions were expressed in *Pichia pastoris* and used to track the toxin's transit through the endocytic pathway, including TIRF microscopy for quantitative analyses of Erd2-GFP mobility in wild-type yeast and selected endocytic mutants.
Our studies were extended by investigating uptake and endocytic transport of the plant A/B toxin ricin. This heteromeric glycoprotein belongs to the family of ribosome inactivating proteins (RIPs) whose in vivo toxicity results from the depurination of 28S rRNA catalyzed by the A-chain of ricin, RTA. Since extention of RTA by a mammalian-specific ER retention signal (KDEL) significantly increases RTA toxicity against mammalian cells, we analyzed the phenotypic effect of RTA carrying the yeast-specific ER retention motif HDEL. Interestingly RTA^{HDEL} showed a similar cytotoxic effect on yeast as a corresponding RTA^{KDEL} variant on HeLa cells. Furthermore, we established a powerful yeast bioassay for RTA in vivo uptake and trafficking. The assay verified the RTA resistant phenotype seen in yeast mutants defective in early steps of endocytosis(Δ*end3*) and/or in RTA depurination activity(Δ*rpl12B*) [3]. Thus K28 and RTA represent powerful tools and substrates for general studies of endocytosis and endosomal trafficking in eukaryotic cells.
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YEV3-FG

The conjunction of mRNA export and translation

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In recent years it has been shown that some mRNA export factors are also involved in translation. Here we report on the identification of a novel transport function of the yeast mRNA export factor Npl3 in the export of large ribosomal subunits from the nucleus to the cytoplasm. Interestingly, while mRNAs are exported via the RNA-binding protein Npl3 and its interacting export receptor Mex67, the export of large ribosomal subunits also requires Mex67, however, in this case Mex67 directly binds to the 5S rRNA and does not require the Npl3-adapter. We discovered a novel function of Npl3 in mediating pre-60S ribosomal subunit export independent of Mex67. Npl3 interacts with the 25S rRNA, ribosomal and ribosome associated proteins and with the NPC. Mutations in *NPL3* lead to export defects of the large subunit and genetic interactions with other pre-60S export factors.

YEV4-FG

Localization of mRNAs and endoplasmic reticulum in budding yeast

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Localization of mRNAs contributes to generation and maintenance of cellular asymmetry, embryonic development and neuronal function [1]. It is a widely distributed process in single-celled and multicellular eukaryotes but has also been described for prokaryotes. In the budding yeast *Saccharomyces cerevisiae*, a minimal protein complex comprised of the myosin motor Myo4p, the RNA binding protein She2p, and the adapter and RNA binding protein She3p localizes >30 mRNAs to the bud tip [2]. This set of mRNAs includes 13 mRNAs encoding membrane or secreted proteins. It has been observed that ribonucleoprotein (RNP) particles containing one of these mRNAs can co-localize with tubular ER structures. Such ER tubules form the initial elements for segregation of cortical ER (cER) [3]. Co-localization has therefore been suggested to illustrate a coordination of mRNA localization and cER distribution [4]. By investigating mRNA localization in yeast mutants defective in cER segregation, we demonstrate that proper cER segregation is required for localization of a subset of mRNAs. These mRNAs are expressed at the time of tubular ER movement into the bud. Localization of *ASH1* mRNA that is expressed after tubular movement has ceased is not affected in any of these mutants. Co-localization of RNPs and tubular ER depends on the RNA-binding protein She2p and requires its tetramerization. She2p can bind to artificial, protein-free liposomes in a membrane curvature-dependent manner with a preference for small liposomes with a diameter resembling yeast ER tubules. In support of this finding, loss of proteins required for tubule formation result in defective mRNA localization *in vivo*. Our results demonstrate that She2p is not only an RNA- but also lipid-binding protein that recognizes membrane curvature, which makes it an ideal coordinator of ER tubule and mRNA co-transport
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YEV5-FG

Eukaryotic Ribosome Biogenesis: Analysis of the Nucleolar Essential Yeast Nep1 Protein and Mutations Causing the Human Bowen-Conradi Syndrome

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In eukaryotes, ribosome biogenesis needs the coordinated interaction of rRNAs and proteins. We identified the Nep1 (Emg1) protein family as an essential protein involved in ribosome biogenesis. The yeast and the human Nep1 proteins are localized in the nucleolus and the human *Hs*Nep1 can complement the Nep1 function in a yeast *Δnep1* mutant.
A mutation which abolished the yeast Nep1 RNA binding was responsible for the human Bown-Conradi-Syndrome (BCS) which causes early child death. Analysis of yeast and human mutations showed that the mutated proteins lost their nucleolar location and their RNA-binding activity.
Structure analysis of the Nep1 protein suggested its function as a methyl transferase and, recently, we could confirm that Nep1 methylated ψ1191 in the decoding center of the 18S rRNA. Additionally, the Nep1 protein has a dual function in ribosome biogenesis and supports Rps19 assembly to the pre-ribosome.
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YEV6-FG

High-level production of tetraacetyl phytosphingosine (TAPS) by combined genetic engineering of sphingoid base biosynthesis and L-serine availability in the non-conventional yeast *Pichia ciferrii*

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The non-conventional yeast *Pichia ciferrii* (formerly known as *Hansenula ciferri*) is the only known organism that is specialized in producing and secreting large quantities of tetraacetyl phytosphingosine (TAPS), a fully acetylated form of the sphingolipid intermediate phytosphingosine. Because of its unique feature this yeast is an attractive microorganism for the industrial production of TAPS. Sphingolipids are important ingredients in cosmetic applications and formulations. They play important roles in human *stratum corneum* as they are involved in skin permeability and antimicrobial barrier homeostatic functions.
Our work aimed to improve TAPS production by genetic engineering of *P. ciferrii*. In a first step, we could increase TAPS production by improving precursor availability. This was achieved by blocking degradation of L-serine which - in the first committed step of sphingolipid biosynthesis - is condensed with palmitoyl-CoA by serine palmitoyltransferase. Moreover, genetic engineering of the sphingolipid pathway further increased secretion of TAPS considerably. The final recombinant *P. ciferrii* strain produced up to 199 $mg_{(TAPS)} * g^{-1}_{(cdw)}$ with a maximal production rate of 8.42 mg * OD_{600nm}^{-1} * h^{-1} and a titer of about 2 g * L^{-1}, and should be applicable for industrial TAPS production.
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YEV7-FG

The genetics of ester synthesis in *Hanseniaspora uvarum* during winemaking

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It is well known that the so called Non-Saccharomycetes have a strong influence on the chemical composition and the sensorical quality of wines. *Hanseniaspora uvarum* is a common yeast found in the early stages of spontaneous wine fermentations but also sometimes in starter cultures inoculated fermentations. Depending on the phytosanitary status of the grapes in a vineyard during ripening up to 90% of a yeast population at the beginning of fermentation can belong to this species. In some cases, fermentations with strains of this organism show interestingly positive bouquets affected by positive esters flavours. But it is also known that fermentations with high amounts of *H. uvarum* during the beginning can be characterized by high amounts of acidic acid and the resulting ethyl acetate ester, both typical off flavours in wine.
The composition and pH of musts, availability of nutrients for yeast growth, the temperature during fermentation and viticultural and oenological methods are parameters which can influence different pathways of the yeast metabolism involved in the production of flavours and aromas, for example esters (Lilly et al., 2000)
In *S. cerevisiae*, esters are produced by two alcohol acyltransferases ATF1 and ATF2 and an acyl-coenzyme A: ethanol O-acyltransferase EEB1. These enzymes and genes are well characterized.
The question is in which metabolic pathway(s) are esters produced by *H. uvarum* and how potential genes are regulated to show the formerly described observations concerning the ester production in a positive or negative way.
In contrast to *S. cerevisiae* genomic data of *H. uvarum* were not available so far. In a cooperation project with J. Heinisch, Department of Genetics, University of Osnabrück, a type strain was sequenced. Using these sequences possible candidates of ATF and EEB genes in *H. uvarum* were identified. Derived primers were used to amplify these genes by PCR. The PCR products were characterized by sequencing and cloned in *E. coli*. One part of the work was to reconstitute the corresponding EuroScarf knock-out-mutants and aftergrape must fermentations the ester formation by analyzed by GC-MS. Another part of this work was to observe under which conditions high amounts of several ester compounds are produced. The next steps will be the development of an efficient transformation protocol, the generation of knock-out and over expression mutants and the analysis of the promotor sequences.

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YEV8-FG

Feel me, thrill me, kill me - when *K. lactis* meets *S. cerevisiae*

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Recent studies have shown that transfer RNAs (tRNAs) are not only essential for decoding messenger RNAs (mRNAs) but also serve as pathorelevant targets for microbial endoribonuclease toxins (ribotoxins) from bacteria, yeast and fungi that cleave within tRNA anticodons and thereby inhibit growth of sensitive target cells. Strikingly, these tRNase ribotoxins ensure survival of their producers against other microbial competitors in the same ecological niche and often, their attacks on tRNAs lead to cell death by way of tRNA depletion. Antifungal tRNase ribotoxins include the zymocin complex from dairy yeast *Kluyveromyces lactis* which kills sensitive cells of baker's yeast *Saccharomyces cerevisiae*. Intriguingly, zymocin's tRNase activity targets tRNA species that possess specific nucleobase modifications at their anticodon wobble position and these modifications are functionally conserved among prokaryal and eukaryal organisms. Therefore, our idea was to take the basic biology of tRNase ribotoxins and apply this to cell systems, including HeLa tumour cells, whose proliferation heavily relies on proper tRNA functions in mRNA translation and *de novo* protein synthesis. Our pilot findings indicate that expression of the zymocin tRNase from *K. lactis* not only kills sensitive cells of *S. cerevisiae* but also affects the growth and viability of higher eukaryal cells including plants and mammals. Hence, we conclude and propose in this session that microbial tRNase ribotoxins may be invoked as novel anti-proliferative factors for biomedical or agrobiological intervention schemes [1].

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Habilitationen

Stephan Seiler habilitierte sich im Januar 2011 an Universität Göttingen (Signaltransduktion und Zellmorphogenese in filamentösen Askomyceten).

Marc Bramkamp habilitierte sich am 27. Januar 2011 an der Universität zu Köln (Temporal and spatial control of cell division in Gram positive, rod-shaped bacteria).

Imke Wiedemann habilitierte sich am 25. Mai 2011 an der Universität Bonn (Die Zellwandvorstufe LipidII – Eine Zielstruktur für Antibiotika).

Rudolf Hausmann habilitierte sich am 5. Juli 2011 an der Universität Karlsruhe (Strategien zur biotechnologischen Produktion von Rhamnolipiden – umweltfreundlichen mikrobiellen Biotensiden).

Darío Ortiz de Orué Lucana habilitierte sich am 7. Juli 2011 an der Universität Osnabrück (Sensing mediated by the novel *Streptomyces* three-component system HbpS-SenS-SenR).

Johannes Gescher habilitierte sich am 7. Juli 2011 an der Universität Freiburg (Metal Respiration / On the components that define a respiratory chain to the cell surface in *Shewanella oneidensis)*.

Ivan Berg habilitierte sich am 7. Juli 2011 an der Universität Freiburg (Diversity of autotrophic carbon fixation and acetyl-coenzyme A assimilation pathways).

Gesche Braker (MPI Marburg) habilitierte sich am 23. November 2011 an der Universität Marburg für das Fachgebiet Mikrobiologie (Ökologie denitrifizierender Lebensgemeinschaften – Einflüsse, Struktur und Funktion).

Sonja-Verena Albers (MPI Marburg) habilitierte sich am 14. Dezember 2011 an der Universität Marburg für das Fachgebiet Mikrobiologie (The archaeal surface).

Rufe angenommen

Marc Thilo Figge von der Universität Frankfurt am Main übernahm am 1. Januar 2011 die W2-Professur Angewandte Systembiologie an der Universität Jena und die Leitung der Forschungsgruppe Angewandte Systembiologie am Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut.

Vera Meyer von der Universität Leiden übernahm am 1. März 2011 die W3 Professur für den Lehrstuhl Angewandte und Molekulare Mikrobiologie an der Technischen Universität Berlin.

Johannes Gescher von der Universität Freiburg übernahm am 1. April 2011 die W3-Professur für den Lehrstuhl Angewandte Biologie am Karlsruher Institut für Technologie.

Peter Graumann von der Universität Freiburg nahm im Mai 2011 einen Ruf an die Universität Marburg an und wird dort im April 2012 beginnen.

Lars Blank von der TU Dortmund übernahm am 1. Juli 2011 eine W3-Professur als Leiter des Instituts für Angewandte Mikrobiologie an der RWTH Aachen.

Martin Thanbichler vom Max-Planck-Institute für terrestrische Mikrobiologie in Marburg übernahm am 5. Juli 2011 eine W2-Professur für Mikrobiologie an der Universität Marburg.

Marcel Kuypers, Direktor am Max-Planck-Institut für Marine Mikrobiologie, nahm im Juli 2011 den Ruf zum Professor für Biogeochemie im Fachbereich Geologie der Universität Bremen an.

Katharina Pahnke von der Universität von Hawaii in Manoa übernahm am 1. August 2011 die W2-Gruppenleitung der Max-Planck-Research Group „Marine Isotope Geochemie“ am Institut für Chemie und Biologie des Meeres (ICBM) der Universität Oldenburg.

Michael Rother von der Universität Frankfurt am Main übernahm am 1. Oktober 2011 die W2-Professur für Mikrobielle Diversität an der Technischen Universität Dresden.

Kürsad Turgay von der FU Berlin übernahm am 1. Oktober 2011 die W2- Professur für Mikrobiologie an der Universität Hannover.

Katharina Riedel von der Technischen Universität Braunschweig übernahm am 1. Oktober 2011 eine W3-Professur am Lehrstuhl für Mikrobiologie an der Universität Greifswald.

Anke Becker von der Universität Freiburg übernahm am 1. Oktober 2011 eine W3-Professur für Mikrobiologie an der Universität Marburg in Verbindung mit dem LOEWE-Zentrum für Synthetische Mikrobiologie (SYNMIKRO).

Bodo Philipp von der Universität Konstanz übernahm am 1. Oktober 2011 die W2-Professur für Mikrobielle Biotechnologie am Institut für Molekulare Mikrobiologie und Biotechnologie der Universität Münster.

Sabine Hunke von der Humboldt-Universität zu Berlin übernahm am 1. Oktober 2011 die Juniorprofessur im Bereich Mikrobiologie am Fachbereich Biologie/Chemie der Universität Osnabrück.

Christoph Mayer, Heisenberg-Stipendiat der Universität Konstanz, übernahm am 1. Oktober 2011 eine Akademische Ratsstelle im Bereich Mikrobiologie und Biotechnologie / Glykobiologie an der Universität Tübingen.

Fördergeld für Bakterien in der Arktis

Prof. Dr. Antje Boetius, Leiterin der Tiefsee-Forschungsgruppe am Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gemeinschaft und Professorin für Geomikrobiologie an der Universität Bremen, erhält vom Europäischen Forschungsrat 3,4 Millionen Euro Fördergelder. Damit will die renommierte Bremer Biologin in den kommenden fünf Jahren den Meeresboden in der arktischen Tiefsee untersuchen und den Geheimnissen seiner rätselhaften Bakterienwelt auf die Spur kommen.

Das Forschungsprojekt „ABYSS – Assessment of bacterial life and matter cycling in deep-sea surface sediments“ setzt auf die Kooperation verschiedener Forschungseinrichtungen in Bremen, aber auch im Ausland. Die Forschungen beginnen bereits dieses Jahr. Einen Großteil der Untersuchungen wird Boetius auf See und am Tiefsee-Observatorium des Alfred-Wegener-Instituts vornehmen: „Wir müssen zu den Bakterien hinabtauchen, denn die meisten lassen sich nicht im Labor kultivieren“, so die Wissenschaftlerin.

„Jedes Gramm Schlamm aus der Tiefsee enthält bis zu 10.000 Arten Mikroorganismen, von denen die meisten unbekannt sind“, schildert Boetius. „Viele sind wahre Hungerkünstler und können aus jedem noch so kleinen Algenrest Energie gewinnen und Biomasse aufbauen. Wie sie das machen, ist ein Rätsel und von großer Bedeutung für den globalen Kohlenstoffkreislauf, die geologischen Ablagerungsprozesse und die Vielfalt des Lebens am Meeresboden“.

Eckhard Boles von der Universität Frankfurt am Main nahm im November 2011 ein Bleibeangebot auf eine W3-Professur am Institut für Molekulare Biowissenschaften an.

Marc Bramkamp von der Universität zu Köln nahm am 27. November 2011 den Ruf auf eine W2-Professur für Mikrobiologie an der Ludwig-Maximilians-Universität München an.

Rolf Daniel von der Universität Göttingen übernahm am 1. Dezember 2011 die W3-Professur Angewandte und Genomische Mikrobiologie an der Universität Göttingen.

Reinhard Guthke wurde am 15. Dezember 2011 auf eine außerplanmäßige Professur Systembiologie an der Universität Jena berufen.

Emeritierungen/ Pensionierungen

Georg Fuchs vom Institut für Mikrobiologie der Universität Freiburg wurde am 31. März 2011 emeritiert.

Alasdair M. Cook vom Fachbereich Biologie an der Universität Konstanz wurde am 31. März 2011 pensioniert.

Bo Barker Jørgensen, Direktor am Max-Planck-Institut für Marine Mikrobiologie, wurde am 30. September 2011 emeritiert.

Rudolf Eichenlaub vom Institut für Gentechnologie/Mikrobiologie an der Universität Bielefeld wurde am 30. September 2011 emeritiert.

Wissenschaftliche Preise 2011

(sofern nicht bereits in BIOspektrum gemeldet)

Susanne Fetzner von der Universität Münster erhielt am 7. Januar 2011 den Forschungspreis der Universität Münster für ihre Arbeiten über Reaktionsmechanismen ringspaltender bakterieller Dioxygenasen.

Marcel Thön vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – erhielt am 14. April 2011 den Wissenschaftspreis für Lebenswissenschaften und Physik des Beutenberg-Campus Jena e.V. für seine Arbeiten zum Thema „Redox regulation of the *Aspergillus nidulans* CCAAT-binding factor (AnCF)".

Holger Zinke von der BRAIN AG wurde am 15. Juni 2011 für seine Beiträge auf dem Gebiet der Biologisierung von Industrien und sein unternehmerisches Engagement mit dem IBN-Award des Vereins Industrielle Biotechnologie Nord ausgezeichnet.

Tamas Dolowschiak von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, erhielt am 01.07.2011 den HIRSIB-Preis für seine Arbeit „Cell-cell communications as essential component of the epithelial innate host defence".

Jutta Vogelmann von der Universität Tübingen erhielt am 21. Juli 2011 den Promotionspreis der Mathematisch-Naturwissenschaftlichen Fakultät für ihre Arbeiten über „Biochemische und biophysikalische Analyse des DNA-Translokator-Proteins TraB des konjugativen Plasmids pSVH1 aus *Streptomyces venezuelae*".

Christian Hertweck von der Universität Jena und vom Leibniz Institut für Naturstoff-Forschung und Infektionsbiologie, Hans-Knöll-Institut (HKI), erhielt am 16. August 2011 den NPR Lecture Award für seine Arbeiten zur Biosynthese mikrobieller Wirkstoffe.

Stephan Fuchs von der Universität Greifswald erhielt am 25. September 2011 den Doktorandenpreis der DGHM für seine Arbeiten über Physiologische und molekularbiologische Untersuchungen zur Adaptation von *Staphylococcus aureus* an anerobe Bedingungen.

Jörg Vogel von der Universität Würzburg erhielt am 25. September 2011 den Hauptpreis der Deutschen Gesellschaft für Hygiene und Mikrobiologie für seine Arbeiten über kleine RNAs in pathogenen Bakterien und wurde im Oktober 2011 auf Lebenszeit als Mitglied bei EMBO (European Molecular Biology Organisation) gewählt.

Matthias Brock vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut – erhielt am 27. Oktober 2011 den Pettenkofer-Preis für seine Arbeiten über „Pilzinfektionen bei abwehrgeschwächten Patienten: Pathogenese, Diagnostik, Therapie und Prävention".

Markus Bröcker von der Universität Yale erhielt am 4. November 2011 den Heinrich-Büssing-Preis der Stiftung zur Förderung der Wissenschaften an der Technischen Universität Braunschweig für seine Promotion zum Thema „Function and Structure of the Light-Independent Protochlorophyllide Oxidoreductase"

Cynthia M. Sharma vom Zentrum für Infektionsforschung der Universität Würzburg erhielt am 04. November 2011 den Ingrid-Zu-Solms Naturwissenschaftspreis 2011 für ihre Dissertation über das Magenbakterium *Helicobacter pylori* sowie am 11. November 2011 den Robert-Koch-Postdoktorandenpreis für Mikrobiologie 2011.

Cecilia Chassin von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, erhielt am 11.11.2011 den Postdoktorandenpreis der Robert-Koch-Stiftung für ihre Arbeit über „miR-146a mediates protective innate immune tolerance in the neonate intestine".

Hans-Wilhelm Nützmann und Kirstin Scherlach vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll erhielten am 7. November 2011 den medac-Forschungspreis für ihre Arbeiten zum Thema „Bacteria-induced natural product formation in the fungus Aspergillus nidulans requires Saga/Ada-mediated histone acetylation".

Volker Schroekh, Fabian Horn und Julia Schümann vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll erhielten am 7. November 2011 den medac-Forschungspreis für ihre Arbeiten zum Thema „Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation".

Qian Chen und Andrea Hartmann vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll erhielten am 7. November 2011 den medac-Forschungspreis für ihre Arbeiten zum Thema „Combined C3b and Factor B autoantibodies and MPGN2".

Judith Klatt vom Max-Planck-Institut für Marine Mikrobiologie in Bremen erhielt am 10. November 2011 in Berlin den nationalen Förderpreis aus dem UNESCO-L'Oréal-Förderprogramm *For Women in Science* als exzellente Doktorandin mit Kind u. a. für ihre Expeditionen zur Erforschung der Bakterienpopulationen an den Sulfidquellen vor den Grotten von Frasassi, Italien.

Ein Team Bielefelder Studenten (**Timo Wolf, Michael Limberg, Jan Schwarzhans, Simon Schäper, Anna Drong, Christian Rückert, Panagiotis Papavasiliou, Dominik Cholewa, Nils Lübke, Maurice Telaar, Robert Braun, Jonas Aretz, Manuel Wittchen, Armin Neshat, Katharina Thiedig, Niko Kessler, Matthias Eder**) erhielt beim Internationalen iGEM-Wettbewerb in Boston, MA, USA einen Gold Award, nachdem sie bereits die Europa-Ausscheidung und damit die Finalteilnahme in Boston gewonnen hatten.

Promotionen 2011

Universität Bayreuth

Kelly Leite: Construction of an efficient secretion system for recombinant proteins in *Bacillus subtilis*
Betreuer: Wolfgang Schumann

Quynh Anh Nguyen: Development of *Bacillus subtilis* spores and cells for surface display of proteins
Betreuer: Wolfgang Schumann

Freie Universität Berlin

Christina Pesavento: Inverse Koordination von Motilität und Genereller Stressantwort in *Escherichia coli*
Betreuerin: Regine Hengge

Tim Kolmsee: Translationale Regulation der SigmaS–Untereinheit der RNA-Polymerase in *Escherichia coli*
Betreuerin: Regine Hengge

Mohammad Rawway Khlaf: Experimental evolution of halotolerance in *Escherichia coli*
Betreuer: Rupert Mutzel

Elisabeth Hauser: Gefahrenidentifizierung der im Schwein epidemiologisch bedeutenden *Salmonella enterica* subsp. *enterica* Serovare 4,[5],12:i:- und Derby
Betreuer: Bernd Appel (Bundesinstitut für Risikobewertung), Rupert Mutzel

Susann Dupke: Untersuchung der Virulenz *Bacillus anthracis*-ähnlicher Isolate aus West- und Zentralafrika
Betreuer: Roland Grunow (Robert Koch-Institut), Rupert Mutzel

Humboldt-Universität zu Berlin

Tobias Goris: Der Einfluss eines neuartigen Fe-S Clusters auf die O_2-Toleranz der membrangebundenen Hydrogenase aus *Ralstonia eutropha*
Betreuerin: Bärbel Friedrich

Volker Müller: The role of the Cpx two-component system in the invasion of *Salmonella enterica* serovar Typhimurium
Betreuer: Erwin Schneider

Technische Universität Berlin

Jean Paul Ouedraogo: The elucidation of signalling and survival mechanisms of fungi to counteract the antifungal protein AFP
Betreuerin: Vera Meyer

Marco Albrecht: Global transcriptome Analysis of the Human Pathogens *Chlamydia trachomatis* and *Chlamydia pneumonia*
Betreuer: Roland Lauster

Universität Bielefeld

Daniella Karine Cavalcanti de Lucena: Characterization of Sigma Factors of *Sinorhizobium meliloti* involved in Stress Response
Betreuer: Alfred Pühler

Anh Vu Nguyen: Time Course Microarray Study of Bio-Hydrogen Production under Sulfur Starvation in the Green Alga *Chlamydomonas reinhardtii*
Betreuer: Olaf Kruse

Johannes Wittmann: Die Endolysine von *Clavibacter michiganensis*-Phagen als Kandidaten für den biologischen Pflanzenschutz von Tomatenpflanzen
Betreuer: Rudolf Eichenlaub

Charlott Sterthoff: *Clostridium botulinum* in der Landwirtschaft und in der Biogasproduktion: Funktionale Charakterisierung eines neuen C2-Toxins und Metagenomik eines Gärrestes
Betreuer: Andreas Tauch

Matthias Keck: Isolierung und Strukturanalyse äußerer Membranlipide von *Sorangium cellulosum* So ce56
Betreuer: Karsten Niehaus

Marcus Persicke: Etablierung und Weiterentwicklung der Metabolomanalyse zur Untersuchung von Aminosäure-produzierenden *Corynebacterium glutamicum* Stämmen
Betreuer: Alfred Pühler

Jens Plassmeier: Funktion und Regulation des Propionatstoffwechsels in *Corynebacterium glutamicum*
Betreuer: Alfred Pühler

Universität Bochum

Sina Langklotz: Substrate diversity and substrate selection of the bacterial FtsH and Lon proteases
Betreuer: Franz Narberhaus

Alexandra Müller: Molybdän-Aufnahme und Mo-abhängige Genregulation in *Rhodobacter capsulatus*
Betreuer: Franz Narberhaus

Ina Wilms: Small regulatory RNAs in *Agrobacterium tumefaciens*
Betreuer: Franz Narberhaus

Andrea Busch: Biosynthesis of phycoerythrobilin and its attachment to phycobiliproteins
Betreuerin: Nicole Frankenberg-Dinkel

Christian Schäfers: Heterotrimere G-Proteine in Pilzen: Die Funktion der Gα-Untereinheit GSA1 in der Entwicklung des Modellorganismus *Sordaria macrospora*
Betreuer: Ulrich Kück

Katarina Kopke: Entwicklung molekulargenetischer Techniken zur Anwendung bei biotechnologisch relevanten Hyphenpilzen
Betreuer: Ulrich Kück

Stefan Gesing: Vergleichende Genexpressionsuntersuchungen bei der Entwicklung von Hyphenpilzen
Betreuerin: Minou Nowrousian

Universität Bonn

Christian Krätzer: Substratumsetzung und Schutz vor Sauerstoffradikalen in *Methanosarcina mazei*
Betreuer: Uwe Deppenmeier

Cornelia Welte: Ferredoxin-dependent electron transport during methanogenesis from acetate
Betreuer: Uwe Deppenmeier

Anne Korsten: Das seltene kompatible Solut N-Acetyl-glutaminylglutamin-1-amid (NAGGN): Heterologe Expression des Genclusters aus *Pseudomonas putida* und Untersuchungen zur Funktion der putativen Biosyntheseenzyme
Betreuer: Erwin A. Galinski

Andrea Meffert: Die Hydroxylierung von Ectoin und Derivaten durch die Hydroxylase EctD aus *Halomonas elongata*
Betreuer: Erwin A. Galinski

Technische Universität Braunschweig

Stefanie Hebecker: Alanyl-Phosphatidylglycerol Synthase from *Pseudomonas aeruginosa*: Physiological relevance and mechanism of tRNA-dependent catalysis
Betreuer: Dieter Jahn

Nathalie Rosin: Physiologie von *Pseudomonas aeruginosa* unter Harnwegs-ähnlichen Bedingungen
Betreuer: Dieter Jahn

Anna-Lena Kaufholz: Aminolevulinic acid synthase of *Rhodobacter capsulatus*
Betreuer: Dieter Jahn

Maike Narten: Charakterisierung der Antibiotikaresistenzmechanismen von *Pseudomonas aeruginosa* unter Harnwegs-ähnlichen Bedingungen
Betreuer: Dieter Jahn

Sonja Storbeck: Structure and Function of *Pseudomonas aeruginosa* NirE involved in heme d_1 biosynthesis
Betreuer: Gunhild Layer

Johannes Walther: Biosynthese des Isobacteriochlorins Häm d_1: Charakterisierung der Proteine NirJ und NirDLGH aus *Pseudomonas aeruginosa*
Betreuer: Gunhild Layer

Lars Remus: Strategien gegen mikrobielle Kontaminationen von Lackieranlagen in der Automobilindustrie
Betreuer: Dieter Jahn

Christopher Untucht: Blut-Hirnschranke-Modelle ECV304-C6 und HBMEC und ihre Anwendung in Transmigrationsuntersuchungen von Streptokokken und afrikanischen Trypanosomen
Betreuer: Michael Steinert

Frank Uliczka: Regulation and function of adhesion and invasion factors of enteropathogenic *Yersinia* species
Betreuerin: Petra Dersch

Anna-Katharina Wagner: Regulation des Virulenzgenregulators SlyA in pathogenen und apathogenen *Escherichia coli*-Stämmen
Betreuerin: Petra Dersch

Katharina Herbst: The temperature- and growth phase-dependent regulation of the global virulence regulator RovA from *Yersina pseudotuberculosis*
Betreuerin: Petra Dersch

Benjamin Stielow: Taxonomy, axenic cultivation, cryopreservation and pathogens of sequestrate macrofungi
Betreuer: Peter Hoffmann

Universität Bremen und MPI für Marine Mikrobiologie, Bremen

Jana Milucka: Dissimilatory sulfur metabolism coupled to anaerobic oxidation of methane
Betreuer: Rudolf Amann

Sabine Lenk: Molecular ecology of key organisms in sulfur and carbon cycling in marine sediments
Betreuer: Rudolf Amann

Kyoko Kubo: Autecology of crenarchaeotal and bacterial clades in marine sediments and microbial mats
Betreuer: Rudolf Amann

Mariette Kassabgy: Diversitiy and abundance of gammaproteobacteria during the winter-spring transition at station Kabeltonne (Helgoland)
Betreuer: Rudolf Amann

Jörg Brock: Impact of sulphide-oxiding bacteria on the phosphorus cycle in marine sediments
Betreuerin: Heide Schulz-Vogt

Verena Salman: Diversity studies and molecular analyses with single cells and filaments of large, colorless sulphur bacteria
Betreuerin: Heide Schulz-Vogt

Anne Schwedt: Physiology of a marine *Beggiatoa* strain and the accompanying organism *Pseudovibrio* sp. – a facultatively oligotrophic bacterium
Betreuerin: Heide Schulz-Vogt

Carsten Frank: Polyphasische Taxonomie, Kerngenom und Lebenszyklus von *Rhodopirellula*-Stämmen
Betreuer: Friedrich Widdel

Ulrike Jaekel: Anaerobic oxidation of short-chain and cyclic alkanes by sulphate-reducing bacteria
Betreuer: Friedrich Widdel

Dennis Fink: Dynamics of Symbiont Abundance in Bathymodiolin Deep-sea Symbioses
Betreuerin: Nicole Dubilier

Frauke Lüddeke: Genetische und biochemische Charakterisierung von Enzymen des anaeroben Monoterpenabbaus in *Castellaniella defragrans*
Betreuer: Friedrich Widdel

Hang Gao: Nitrogen loss in the intertidal permeable Wadden Sea sediments
Betreuer: Marcel Kuypers

Joanna Sawicka: Arctic to tropic – adaptation and response of anaerobic microorganisms to temperature effects in marine sediments
Betreuer: Bo Barker Jørgensen

Kyriakos Vamvakopoulos: Plastifying marine microhabitats – exploring biogeochemical processes in microscale
Betreuer: Bo Barker Jørgensen

Christina Bienhold: Diversity and ecology of bacterial communities at the deep seafloor
Betreuerin: Antje Boetius

Jan Erik Rau: Characterisation of inhibitory substances produced by two *Pseudoalteromonas* species and the cyanobacterial strain Flo 1
Betreuer: Ulrich Fischer

Clelia Dona: Mobilization of sulfur by green sulfur bacteria – Physiological and molecular studies on *Chlorobaculum parvum* DSM 263
Betreuer: Ulrich Fischer

Jan P. Schrübbers: Strategien zur Detektion und Identifizierung von Naturstoffen bei Cyanobakterien am Beispiel von *Geitlerinema* Flo 1 und *Fischerella ambigua*
Betreuer: Ulrich Fischer

Astrid Näther: Phylogenetische und funktionelle Diversität von *Acidobacteria* in Wald- und Grünlandböden unterschiedlicher Landnutzung
Betreuer: Michael W. Friedrich

International Jacobs University Bremen und MPI für Marine Mikrobiologie

Basak Öztürk: Assessing the Genetic Accessibility of *Rhodopirellula baltica* SH 1^t
Betreuer: Frank-Oliver Glöckner

Ivaylo Kostadinov: Marine Metagenomics: From high-throughput data to ecogenic interpretation
Betreuer: Frank-Oliver Glöckner

Wolfgang Hankeln: Data intergration in microbial genomics – contextualizing sequence data in aid of biological knowledge
Betreuer: Frank-Oliver Glöckner

Elmar Prüße: Scaleable bioinformatic methods and resources for ribosomal RNA based studies
Betreuer: Frank-Oliver Glöckner

Pelin Yilmaz: Improving the Usage of Ribosomal RNA Gene in Microbiology and Microbial Ecology – Importance of standardization and biocuration
Betreuer: Frank-Oliver Glöckner

Universität Darmstadt

Andreas Veith: Sulfur oxygenase reductases – a structural and biochemical perspective
Betreuer: Arnulf Kletzin

Technische Universität Dortmund

Jan Heyland: β-Aminopeptidases: Recombinant production and cell based biocatalysis
Betreuer: Andreas Schmid, Lars Blank

Birgitta Ebert: A systems approach to understand and engineer whole-cell redox biocatalysts
Betreuer: Andreas Schmid, Lars Blank

Sjef Cornelissen: Whole-cell biotransformations using cytochrome P450 monooxygenases: potential and limitations
Betreuer: Andreas Schmid, Bruno Bühler

Özde Ütkür: Regioselective aerobic and anaerobic aromatic hydroxylations with molybdenum hydroxylases in *Pseudomonas* sp.
Betreuer: Andreas Schmid, Bruno Bühler

Technische Universität Dresden

Venelina Yovkova: Gentechnische Optimierung der Hefe *Yarrowia lipolytica* für die biotechnologische Gewinnung von α-Ketoglutarsäure
Betreuer: Gerold Barth

Martina Holz: Gentechnische Optimierung der Hefe *Yarrowia lipolytica* zur biotechnologischen Produktion von Succinat
Betreuer: Gerold Barth

Angela Jacobi: Die Rolle der CXCR4/SDF-1-Achse in FLT3-ITD-positiven humanen hämatopoetischen Stammzellen
Betreuer: Gerold Barth

Christine Steinbrenner: Biochemische und molekularbiologische Charakterisierung von Biofilmen des WSB-Verfahrens im Vergleich zum Belebungsverfahren unter besonderer Berücksichtigung der Nitrifikation
Betreuerin: Isolde Röske

Lysann Mehlig: Analyse und Vergleich der Biodiversität in der Gemeinschaft Polyphosphat akkumulierender Mikroorganismen (PAO) an Belebtschlämmen kommunaler Kläranlagen
Betreuerin: Isolde Röske

Universität Duisburg-Essen

Miriam Moritz: Integration of hygienically relevant bacteria in drinking water biofilms grown on domestic plumbing materials
Betreuer: Hans-Curt Flemming

Universität Düsseldorf

Eva M. Szafranski-Schneider: Charakterisierung des Msb2 Sensorproteins in *Candida albicans*
Betreuer: Joachim F. Ernst

Theresia Lassak: Untersuchungen zur Regulation und Bindespezifität des Transkriptionsfaktors Efg1 in *Candida albicans*
Betreuer: Joachim F. Ernst

Universität Erlangen-Nürnberg

Nadja Jeßberger: The GlnR-dependent nitrogen regulatory network of *Mycobacterium smegmatis*
Betreuer: Andreas Burkovski

Katrin Pfeffer: Leader-RNA vermittelte Regulation der TetO-Expression
Betreuer: Andreas Burkovski

Carolin Wagner: Characterization of the *Salmonella* Pathogenicity Island 4-encoded proteins SiiE, SiiA and SiiB: a new mechanism of bacterial adhesion
Betreuer: Michael Hensel (Osnabrück)

Universität Frankfurt am Main

Saskia Köcher: Etablierung eines genetischen Systems für das moderat halophile Bakterium *Halobacillus halophilus*: Mutantenanalysen zur physiologischen Funktion von Carotinoiden und der Osmolyte Prolin und Glutamin
Betreuer: Volker Müller

Eva Biegel: Energy conservation in *Acetobacterium woodii:* Identification and characterization of a Na^+-translocating ferredoxin: NAD^+ oxidoreductase (Rnf) and a Na^+-pyrophosphatase
Betreuer: Volker Müller

Silke Schmidt: Die Entschlüsselung des Genoms von *Acetobacterium woodii:* neue Einblicke in die Lebensweise und Bioenergetik eines acetogenen Bakteriums
Betreuer: Volker Müller

Tilmann Stock: Molekulargenetische Analyse *trans*-aktiver Faktoren der Selenoprotein-Biosynthese in *Methanococcus maripaludis*
Betreuer: Michael Rother

Universität Freiburg

Miriam Kaufenstein: The assembly of the competence machinery in *Bacillus subtilis*
Betreuer: Peter Graumann

Rafael Say: Die Fructose-1,6-Bisphosphat-Aldolase/Phosphatase – ein ursprüngliches gluconeogenetisches Enzym
Betreuer: Georg Fuchs

Tobias Knust: Regulation of SMC by associate proteins and ATP
Betreuer: Peter Graumann

Theresa Bauer: Investigation of pLS20 mediated conjugative DNA transfer in the Gram positive bacterium *Bacillus subtilis*
Betreuer: Peter Graumann

Robin Teufel: Aerobic pheylacetate catabolism – A novel principle of aromatic degradation
Betreuer: Georg Fuchs

Miriam Pediaditakis: Investigations on behaviour and specific localization of DNA repair proteins in *Bacillus subtilis*
Betreuer: Peter Graumann

Liv Rather: Structure and mechanism of benzoyl-CoA epoxidase BoxAB from *Azoarcus evansii*
Betreuer: Georg Fuchs

Universität Gießen

Caroline Knoll: Evaluating the influence of stress parameters on *Oenococcus oeni* and the subsequent volatile aroma composition of white wine
Betreuerin: Sylvia Schnell

Bork Berghoff: The role of small regulatory RNAs in the photooxidative stress response of *Rhodobacter* and *Roseobacter*
Betreuerin: Gabriele Klug

Ramakanth Madhugiri: Messenger RNA stability determinants in *Halobacterium salinarum* NRC-1 & Processing and turn-over of the small non-coding RNA RprA in *Escherichia coli*
Betreuerin: Gabriele Klug

Verena Roppelt: Die Untersuchung der physiologischen Rolle der Exosom-Untereinheiten Rrp4, Csl4 und DnaG aus *Sulfolobus solfataricus*
Betreuerin: Gabriele Klug

Jenny Schäfer: Untersuchungen zur Diversität von *Actinobacteria* in Innenräumen
Betreuer: Peter Kämpfer

Kerstin Fallschissel: Untersuchung an Bioaerosolen in Tierställen unter Etablierung einer REAL-TIME PCR-basierten Methode zur Erfassung luftgetragener *Salmonella* und *Thermoactinomyces* Zellen
Betreuer: Peter Kämpfer

Elena Martin: Luftgetragene Bakterien in der Geflügelwirtschaft – Erfassung der Exposition und Emission
Betreuer: Peter Kämpfer

Universität Göttingen

Van Tuan Tran: Adhesion of the rapeseed pathogen *Verticillium longisporum* to its host *Brassica napus* – Uncovering adhesion genes and the evolutionary origin of the fungus
Betreuer: Gerhard Braus

Anne Dettmann: Regulation and communication between the NDR kinase COT1, the MAK2 MAP kinase cascade and the Striatin complex in *Neurospora crassa*
Betreuer: Gerhard Braus, Stephan Seiler

Christine Diethmaier: Die Rolle von YmdB als Regulator der Zelldifferenzierung in *Bacillus subtilis*
Betreuer: Jörg Stülke

Katrin Gunka: Der Einfluss der Glutamatdehydrogenasen auf die Verknüpfung des Kohlenstoff- und Stickstoffstoffwechsels in *Bacillus subtilis*
Betreuer: Jörg Stülke

Christoph Wrede: Metabolismus und Biomineralisation in anaerob Methan-oxidierenden Lebensgemeinschaften
Betreuer: Michael Hoppert

Martin Lehnik-Habrink: An mRNA degradation complex in *Bacillus subtilis*
Betreuer: Jörg Stülke

Jens Landmann: Das *crh*-Operon in *Bacillus subtilis*: Ein neuartiges Gensystem mit einer zentralen Rolle in Kohlenstoffmetabolismus
Betreuer: Boris Görke

Universität Greifswald

Carmen Wolf: Charakterisierung von *Staphylococcus aureus* Isolaten aus bovinen Mastitisinfektionen und Analyse der oxidativen und nitrosativen Stressantwort
Betreuer: Michael Hecker

Andreas Willy Otto: Mass spectrometry based proteomics approaches unraveling dynamic processes in the entire bacterial cell
Betreuer: Michael Hecker

Alexander Elsholz: Regulation of Protein quality control systems in low GC, Gram-positive bacteria
Betreuer: Michael Hecker

Jan Muntel: Entwicklung und Etablierung von Massenspektrometrie-basierten relativen und absoluten Quantifizierungsmethoden zur physiologischen Proteomanalyse Gram positiver Bakterien
Betreuer: Michael Hecker

Tobias Härtel: Aufklärung grundlegender Aminosäuresynthesewege von *Streptococcus pneumoniae* mittels Isotopolog Profiling und Einfluss des Glutaminmangels auf die bakterielle Virulenz
Betreuer: Sven Hammerschmidt

Universität Halle-Wittenberg

Doreen Koch: Charakterisierung eines neuen, zweiteiligen Eisenaufnahmesystems aus dem uropathogenen *Escherichia coli* Stamm F11
Betreuer: Dietrich H. Nies

Universität Hamburg

Dagmar Krysciak: The complete genome sequence of *Rhizobium* sp. NGR234 reveals a surprisingly large number of quorum quenching associated genes
Betreuer: Wolfgang Streit

Patrick Bijtenhoorn: Characterization of novel metagenomic quorum quenching enzymes attenuating *Pseudomonas aeruginosa* motility, biofilm formation and virulence
Betreuer: Wolfgang Streit

Nele Ilmberger: Metagenomic cellulases linking IL-tolerance, halotolerance and thermostability
Betreuer: Wolfgang Streit

Sabine Keuter: Characterization of nitrifying bacteria in marine recirculation aquaculture systems with regard to process optimization
Betreuerin: Eva Spieck

Technische Universität Hamburg-Harburg

Barbara Klippel: Charakterisierung von cellulolytischen und xylanolytischen Enzymen aus dem thermophilen anaeroben Bakterium *Fervidobacterium gondwanense*
Betreuer: Garabed Antranikian

Vera Bockemühl: Produktion einer Endo-β-1,4-Xylanase aus *Myceliophthora thermophila* mittels Hochzelldichtefermentation von *Pichia pastoris* und Charakterisierung des Enzyms
Betreuer: Garabed Antranikian

Dina Jabbour: A Novel β-Glucosidase and a Chimeric Endoglucanase-CBM for Cellulose Hydrolysis at Elevated Temperatures
Betreuer: Garabed Antranikian

Adriane Lochner: Proteomic characterization of the cellulolytic enzyme system expressed by the extremely thermophilic bacteria *Caldicellulosiruptor* spp.
Betreuer: Garabed Antranikian

Universität Hannover

Denise Mehner: Charakterisierung und Identifizierung eines Interaktionspartners des Tat-Translokons von *Escherichia coli*
Betreuer: Thomas Brüser

Medizinische Hochschule Hannover

Henriette Langhans: The role of the global transcriptional regulator SlyA for the virulence of enteric *Escherichia coli*
Betreuerin: Petra Dersch

Nina Coombs: *Helicobacter pylori* affects ubiquitin-related pathways and components within the human host cell
Betreuerin: Christine Josenhans

Tamas Dolowschiak: Cell-cell communication as essential component of the epithelial innate host defence
Betreuer: Mathias Hornef

Johanna Pott: Epithelial host response to infections of the neonatal enteric mucosa
Betreuer: Mathias Hornef

Universität Hohenheim

Maike Schwidder: Molekulargenetische Untersuchungen zur Expression des Typ III-Effektors NleA4795 von Shiga-Toxin produzierenden *Escherichia coli*
Betreuer: Herbert Schmidt

Helen Stöber: Interaktionen von Milchsäurebakterien mit pathogenen Bakterien und Wirtszellen
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Stefan Roth: Investigations on the mechanism of sterilization by non-thermal low-pressure nitrogen-oxygen plasmas
Betreuer: Christian Hertel

Universität Jena

Tchize Ndejouong Basile Le Sage: Isolation, Structure Elucidation and Biological Evaluation of Metabolites from *Streptomyces* spp. Dwelling in Extreme and Unusual Habitats
Betreuer: Christian Hertweck

Thorger Leif Linke: Isolierung und Strukturaufklärung kryptischer Sekundärmetabolite aus *Burkholderia thailandensis* und *Clostridium cellulolyticum*
Betreuer: Christian Hertweck

Gerald Lackner: Das Genom des endofungalen Bakteriums *Burkholderia rhizoxinica* – molekulare Grundlagen der Bakterien-Pilz-Interaktion
Betreuer: Christian Hertweck

Randa Abdou: Bioactive secondary metabolites from the endophytic microorganisms of the medicinal plant *Bidens pilosa*
Betreuer: Christian Hertweck

Patrícia Bezerra Gomes: Sekundärmetabolite von Actinomyceten aus belasteten Innenräumen
Betreuer: Christian Hertweck

Michael Reuter: Die Regulation des Komplementsystems im Rahmen von Infektionen
Betreuer: Peter F. Zipfel

Josephine Losse: Interaktion neutrophiler Granulozyten mit *Candida albicans*: Rolle von Komplementrezeptor-3-Liganden bei der Beeinflussung der Wirtszellantwort
Betreuer: Peter F. Zipfel, Mihály Józsi

Radhika Jain: The Map Kinase A (MpkA) regulated cell wall integrity signaling pathway in the pathogenic fungus *Aspergillus fumigatus*
Betreuer: Axel Brakhage

Martin Vödisch: Anpassung von *Aspergillus fumigatus* an hypoxische Bedingungen: Vergleichende Proteomanalysen und Charakterisierung Hypoxie-regulierter Proteine
Betreuer: Axel Brakhage

Felicitas Schöbel: Lysinbiosynthese von *Aspergillus fumigatus*: Virulenzstudien und biochemische Analysen
Betreuer: Matthias Brock

Lydia Schild: Einfluss zelloberflächenassoziierter Proteasen auf die Zellbiologie und Pathogenität von *Candida albicans*
Betreuer: Bernhard Hube

Betty Wächtler: Molekulare Charakterisierung der Invasionsmechanismen von *Candida albicans* bei oberflächlichen Infektionen
Betreuer: Bernhard Hube

Johannes Wollbold: Attribute Exploration of Gene Regulatory Processes
Betreuer: Reinhard Guthke

Sandro Lambeck: Systembiologische Charakterisierung der organübergreifenden Transkriptomantwort in muriner Sepsis und die Modellierung bimodaler Muster
Betreuer: Reinhard Guthke

Marc Carlsohn: Isolation and characterization of mine-dwelling actinomycetes as potential producers of novel bioactive secondary metabolites
Betreuer: Hans Peter Saluz

Nicole Borth: Funktionelle Charakterisierung neuer Interaktionen zwischen chlamydialen Proteinen und Wirtszellproteinen
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Katrin Volling: The human pathogenic fungus *Aspergillus fumigatus* inhibits apoptosis in alveolar macrophages
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Anindita Sarkar: Characterization of Silent Secondary metabolite Gene Clusters in the filamentous fungi: *Aspergillus nidulans*
Betreuer: Uwe Horn

Forschungszentrum Jülich

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Betreuer: Michael Bott

Abigail Koch-Koerfges: Novel insights into the energy metabolism of *Corynebacterium glutamicum* by comprehensive analysis of mutants defective in respiration or oxidative phosphorylation
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Sabine Krawczyk: Signaltransduktion in *Corynebacterium glutamicum*: Studien zur Rolle der FHA-Proteine
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Boris Litsanov: Metabolic engineering of *Corynebacterium glutamicum* for efficient succinate production
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Stephanie Schelder: CopRS and CsoR: two regulatory systems involved in copper homeostasis of *Corynebacterium glutamicum*
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Stefanie Schweikert: Transcriptional responses and transcriptional regulators of *Gluconobacter oxydans* 621H
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Miriam Müller: Die Regulation des Zweikomponentensystems CiaRH von *Streptococcus pneumoniae* und die phänotypischen Konsequenzen
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Tina Becker: Das ESX-1 Sekretionscluster von *Streptococcus oralis* Uo5
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Universität Karlsruhe – KIT

Sonja Sand: Charakterisierung eines Blaulichtrezeptors in *Alternaria alternata*
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Tobias Schunck: Charakterisierung des Motorproteins KipA und der Einsatz von Kinesinen zur Entwicklung eines Transportsystems im Nanomaßstab
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Jan Siebenbrock: Isolierung und Charakterisierung von Vesikeln aus dem Spitzenkörper von *Aspergillus nidulans* und deren Einsatz in der Nanobiologie
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Tanja Throm: Charakterisierung der Hydrophobine in *Aspergillus nidulans* und deren Anwendung zur Oberflächenbeschichtung und –funktionalisierung
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Nadine Zekert: On the role of the kinesin-3 motor protein UncA and the role of different microtubule populations in the filamentous fungus *Aspergillus nidulans*.
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Birgit Hobl: Konstruktion neuartiger Expressionsvektoren zur Optimierung der Proteinproduktion in der Hefe *Pichia pastoris*
Betreuer: Matthias Mack (Mannheim), Reinhard Fischer

Hannah Kuhn: Identification and characterization of *Medicago truncatula* marker genes for recognition of fungal signals in the arbuscular mycorrhiza symbiosis
Betreuerin: Natalia Requena

Silke Kloppholz: Das Effektorprotein SP7 und seine Rolle in der arbuskulären Mycorrhizasymbiose
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Ibrahim Njimona: Molecular studies on light induced protein conformational changes on *Agrobacterium tumefaciens* phytochrome, Agp1
Betreuer: Tilman Lamparter

Gregor Rottwinkel: Studien zu Verbreitung, Charakteristika und Funktionen der Bakteriophytochrome in *Rhizobiales*
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Benjamin Zienicke: Fluoreszenzeigenschaften und Photokonversion der Modell-Pyhtochrome Agp1 und Agp2 aus *Agrobacterium tumefaciens*
Betreuer: Tilman Lamparter

Katrin Brzonkalik: Process Development for the Production of *Alternaria* Toxins in a Bioreactor
Betreuer: Christoph Syldatk, Clemens Posten

Markus Michael Müller: Optimization and Characterization of Microbial Rhamnolipid Production from Renewable Resources
Betreuer: Christoph Syldatk, Clemens Posten

Berna Gerce: Bacterial Communities of Different Mediterranean Sponge Species – Basic Investigations for Biotechnological Sponge Cultivation
Betreuer: Christoph Syldatk, Ursula Obst

Max Rubner Institut, Karlsruhe

Eva Graf: Vorkommen, Biodiversität und molekulares Monitoring von mykotoxinbildenden *Alternaria*-Spezies in Lebensmitteln
Betreuer: Rolf Geisen

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Carolin Löscher: Sensitivity of the biological oceanic nitrogen cycle to changes in dissolved oxygen
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Imke Schneemann: Nachweis von Biosynthesegenen des bakteriellen Sekundärstoffwechsels sowie Isolierung und Strukturaufklärung von Naturstoffen aus ausgewählten Actinomyceten
Betreuer: Johannes F. Imhoff

Herwig Heindl: Antimicrobially active microorganisms associated with marine bryozoans
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Franz Goecke: Association between microbes and macroalgae: host, epiphyte and environmental effects
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Alexander Henrich: Characterization of Maltose and Trehalose Transport in *Corynebacterium glutamicum*
Betreuer: Reinhard Krämer

Ines Ochrombel: Untersuchungen zu Mechanismen der Stressantwort und des Kaliumtransports in *Corynebacterium glutamicum*
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Betreuer: Bernhard Schink

Carlos H Dullius: Physiology and biochemistry of the anaerobic biodegradation of isopropanol and acetone
Betreuer: Bernhard Schink

Janina Horst: Characterization of the ribosome-associated complex RAC from *S. cerevisiae*
Betreuerin: Elke Deuerling

Steffen Preißler: Insights into cotranslational protein folding and protein quality control systems on ribosomes
Betreuerin: Elke Deuerling

Joachim Schott: Physiology, ecology and biochemistry of anaerobic phototrophic oxidation of nitrite
Betreuer: Bernhard Schink

Vemparthan Suvekbala: Physiology and biochemical diversity of bacterial cholate degradation
Betreuer: Bodo Philipp

Jutta Mayer: Microbial desulfonation pathways for natural and pharmacologically relevant C_3-sulfonates
Betreuer: Alasdair M. Cook

Universität Leipzig

Johannes W. Kung: Identifizierung und Charakterisierung einer neuen Klasse von Benzoyl-CoA Reduktasen
Betreuer: Matthias Boll

Kevin Kuntze: Nachweis und Charakterisierung von Schlüsselenzymen des anaeroben Abbaus halogenierter und nicht-halogenierter aromatischer Verbindungen
Betreuer: Matthias Boll

Universität Mainz

Patrick Sebastian: Identifizierung biogene Amine bildender Bakterien und Einsatz von Enzymen zur Hemmung ihres Wachstums während der Weinbereitung
Betreuer: Helmut König

Pia Dünnwald: PAS_c als signaltransduzierende Domäne des Sensorproteiens DcuS von *Escherichia coli*
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Universität Marburg und MPI für Terrestrische Mikrobiologie, Marburg

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Daniela Kiekebusch: Die *P-loop*-ATPase MipZ: Mechanismus der Bildung eines Proteingradienten in einer prokaryotischen Zelle
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Andrea Möll: Anatomy of the divisome during the late stages of cell division in the asymmetric α-proteobacterium *Caulobacter crescentus*
Betreuer: Martin Thanbichler

Pittelkow, Marco: Synthese und physiologische Funktion der chemischen Chaperone Ectoin und Hydroxyectoin
Betreuer: Erhard Bremer

Susan Schlimpert: About rings and crossbands – Characterization of proteins involved in cell division and compartmentalization in *Caulobacter crescentus*
Betreuer: Martin Thanbichler

Meike Ammon: Analyse der subzellulären Lokalisation des C-Signalvorläuferproteins p25 und die Identifikation der PopC-Spaltstelle in p25 in *Myxococcus xanthus*
Betreuerin: Lotte Søgaard-Andersen

Hassan Ghareeb: Molecular Dissection of Maize-*S. reilianum* Interactions: Host Developmental Changes and Pathogen Effectors
Betreuer: Jan Schirawski (Göttingen)

Julia Gödeke: Molekulare Mechanismen während der Anheftung und Biofilmbildung in *Shewanella oneidensis* MR1 – Die Tücken des Besiedelns
Betreuer: Kai Thormann

Janine Koepke: Die Rolle des RNA-bindenden Proteins Rrm4 während des polaren Wachstums von *Ustilago maydis*
Betreuer: Michael Feldbrügge (Düsseldorf)

Andrea Koerdt: Biofilm formation in the thermoacidophilic crenarchaea *Sulfolobus spp*
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Anna Konovalova: Regulation of secretion of the signalling protease PopC in *Myxococcus xanthus*
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Daniel Lanver: Apressorienbildung von *Ustilago maydis* auf hydrophoben Oberflächen: Regulation durch Membranproteine
Betreuerin: Regine Kahmann

Jennifer Pratscher: Investigation of microbial groups involved in the uptake of atmospheric trace gases in upland soils
Betreuer: Ralf Conrad

Lei Wang: Functional characterization of a seven-WD40 repeat protein Rak1 in *Ustilago maydis*
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Holger Webert: Strukturelle und funktionelle Charakterisierung von Komponenten der eukaryotischen Eisen-Schwefel-Cluster-Biogenese-Maschinerie
Betreuer: Roland Lill

Technische Universität München

Georg Lutterschmid: Surface active proteins from *Fusarium* spp. and their role in gushing of carbonated beverages
Betreuer: Rudi F. Vogel, Martin Krottenthaler

Simone Freiding: Identification of genetic markers and bottlenecks in *Lactobacillus sakei* constituting safety and quality determinants of fermented sausages
Betreuer: Rudi F. Vogel, Siegfried Scherer

Matthias Stübner: Hydrophobins and Fungispumins – surface active fungal proteins with a role in foam stability of carbonated beverages and fungus-plant interaction
Betreuer: Rudi F. Vogel, Ralph Hückelhoven

Patrick Preissler: Categorization of *Lactobacillus brevis* along their beer-spoiling potential
Betreuer: Rudi F. Vogel, Wolfgang Liebl, Elke Arendt

Samir Velagić: Partielle Charakterisierung der antilisteriellen Wirkung von *Pichia norvegensis* und *Staphylococcus equorum*
Betreuer: Siegfried Scherer

Evi Lang Halter: Prävalenz und Biodiversität von *Listeria* Spezies in limnischen Habitaten
Betreuer: Siegfried Scherer

Elrike Frenzel: Regulation of the biosynthesis of the food-borne *Bacillus cereus* toxin cereulide
Betreuer: Siegfried Scherer

LMU München

Tobias Kraxenberger: Zur Funktion des Sensor-Histidinkinase/Antwortregulator Systems YehU/YehT in *Escherichia coli*
Betreuerin: Kirsten Jung

Ina Maria Suntka Haneburger: Insights into the Molecular Signal Perception Mechanism of the Membrane-Integrated Transcriptional Activator CadC *of Escherichia coli*
Betreuerin: Kirsten Jung

Tina Wecke: Extracytoplasmic Function Sigma Factors in *Bacillus* Species: Investigation of Cell Envelope Stress Responses and Novel Signal Transducing Mechanisms
Betreuer: Thorsten Mascher

Dan Li: Phenotypic variation and molecular signaling in the interaction of the rhizosphere bacteria *Acidovorax* sp. N35 and *Rhizobium radiobacter* F4 with roots
Betreuer: Anton Hartmann

Xiao Chen: Development and characterisation of an immunochemical test system for the determination of bacterial signal molecules (N-acylated homoserine lactones)
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Annemarie Hütz: Bacterial chemotaxis in the ultraoligotrophic Eastern Mediterranean Sea
Betreuer: Jörg Overmann

Hong Cheng: Population structure and species description of aquatic *Sphingomonadaceae*
Betreuer: Jörg Overmann

Universität Münster

Björn Andreeßen: Biotechnological conversion of glycerol
Betreuer: Alexander Steinbüchel

Claudia Borgmeier: Applied aspects of extracellular enzyme synthesis regulation in industrially relevant *Bacilli* for strain optimization focussing on the DegSU system
Betreuer: Friedhelm Meinhardt

Veronika Deppe: Degradation of Amadori products in *Bacillus subtilis*: The physiological relevance and transcriptional regulation of the *frl BONMD* operon
Betreuer: Friedhelm Meinhardt

Kerstin Hoffmann: Genetic and molecular biological analysis of natural genetic competence in *Bacillus licheniformis* and strain improvement
Betreuer: Friedhelm Meinhardt

Chlud Kaddor: *Ralstonia eutropha* H16: a PTS view to the genome
Betreuer: Alexander Steinbüchel

Nicole Lange: Biotechnologische Produktion von Terpenen mit rekombinanten Stämmen von *Saccharomyces cerevisiae*
Betreuer: Alexander Steinbüchel

Kaichien Lin: Studies on cyanophycin produced by recombinant bacteria and the related enzymes
Betreuer: Alexander Steinbüchel

Matthias Raberg: A proteomic view on *Ralstonia eutropha*'s versatile physiology
Betreuer: Alexander Steinbüchel

Universität Oldenburg

Sabrina Beckmann: Microbial Methane formation in abandoned coal mines in the Ruhr Basin of Germany
Betreuer: Heribert Cypionka

Jutta Graue: Bioreactor Janssand: fermentation processes in tidal-flat sediments of the German North Sea
Betreuer: Heribert Cypionka

Daniela Kalhöfer: Genome analysis and comparative genomics of host-associated bacteria of the marine *Roseobacter* clade
Betreuer: Meinhard Simon

Dagmar Rocker: Bakterieller Abbau von refraktärem organischem Material im Weserästuar und in der Nordsee
Betreuer: Meinhard Simon

Kathleen Trautwein: Physiological and proteomic response of *Aromatoleum aromaticum* EbN1 to substrate mixtures and carbon limitation
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Universität Osnabrück

Wael Abdel Halim Hegazy: Optimization of *Salmonella enterica* as live vaccine carrier,
Betreuer: Michael Hensel

Universität Potsdam

Lars Ganzert: Bacterial diversity and adaptation in permafrost-affected soils of maritime Antarctica and Northeast Greenland
Betreuer: Dirk Wagner

Universität Regensburg

Ulf Küper: Untersuchungen zur Energiegewinnung des hyperthermophilen, schwefelreduzierenden Archaeons *Ignicoccus hospitalis*
Betreuer: Harald Huber, Reinhard Rachel

Simone Schopf: Untersuchungen der Flagellen von *Pyrococcus furiosus*: Flagellenvermittelte Interaktionen, Ultrastruktur und Molekularbiologie
Betreuer: Reinhard Wirth

Annett Bellack: Cell Architecture and Flagella of Hyperthermophilic Archaea
Betreuer: Reinhard Wirth

Universität Rostock

Claudia Hackenberg: Photorespiration in Cyanobakterien: funktionelle, regulatorische und evolutionäre Aspekte
Betreuer: Martin Hagemann

Universität des Saarlandes

Esther Gießelmann: Endozytose und intrazellulärer Toxintransport in Hefe- und Säugerzellen mittels fluoreszenzmarkierter K28-Varianten
Betreuer: Manfred Schmitt

Silvia Boschi Bazan: Vergleichende Untersuchungen zur Anwendung verschiedener Hefegattungen als Vehikel für das Protein-Delivery in Antigen-präsentierende Zellen
Betreuer: Manfred Schmitt

Eva Dörrschuck: Molekularbiologische Untersuchungen zur Interaktion der antiretroviral wirkenden porzinen und humanen A3-Proteine mit porzinen endogenen Retroviren (PERV) und Charakterisierung des porzinen A3-Genlokus
Betreuer: Manfred Schmitt

Julia Dausend: *In vivo*-Topologie und Lokalisation des zellulären HDEL-Rezeptors Erd2p und dessen Funktion bei Endozytose und endosomaler Zielsteuerung des viralen K28-Toxins in Hefe
Betreuer: Manfred Schmitt

Mark Lind: Struktur- und Funktionsuntersuchungen von Zygocin – ein kanalbildendes Proteintoxin mit antimykotischem Potential
Betreuer: Manfred Schmitt

Universität Stuttgart

Clarisse Brüning Schmit Roepke: Development of Acetylcholinesterase biosensors for neurotoxins detection in foods and the environment
Betreuer: Bernhard Hauer

Benjamin Juhl: Entwicklung und Anwendung einer flexiblen Dockingmethode für Enzyme und Substrate: Substrate-imprinted docking
Betreuer: Jürgen Pleiss

Evelyne Weber Protein Engineering von P450 Monooxygenasen zur selektiven Hydroxylierung von acyclischen Alkanen
Betreuer: Bernhard Hauer

Sven Rustler: Die enzymatische Hydrolyse von Nitrilen unter sauren Bedingungen durch neu isolierte und rekombinante Mikroorganismen und die Verwendung von säuretoleranten Ganzzellbiokatalysatoren zur enantioselektiven Synthese von alpha-Hydroxycarbonsäuren
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Jiyong Su: Structure-function analysis of a cyanobacterial protein phosphatase 2C homologue
Betreuer: Karl Forchhammer

Oleksandra Fokina: From metabolite sensing to protein regulation by *Synechococcus elongatus* PCC 7942 PII protein
Betreuer: Karl Forchhammer

Susanne Berendt: Funktionelle Charakterisierung von Zellwandproteinen in der Zelldifferenzierung von Heterozysten bildenden Cyanobakterien der Ordnung *Nostocales*
Betreuer: Karl Forchhammer, Iris Maldener

Eva Waldvogel: Aminosäureanalyse von *Streptomyces coelicolor* Mutanten des Stickstoff-Metabolismus und verschiedenen Cyanophycin-Produzenten
Betreuer: Wolfgang Wohlleben

Andrea Heichlinger: Charakterisierung der MreB homologen Proteine während der morphologischen Differenzierung in *Streptomyces coelicolor* A3(2)
Betreuer: Wolfgang Wohlleben

Ewa Musiol: The discrete acyltransferases KirCI and KirCII involved in kirromycin biosynthesis
Betreuer: Wolfgang Wohlleben

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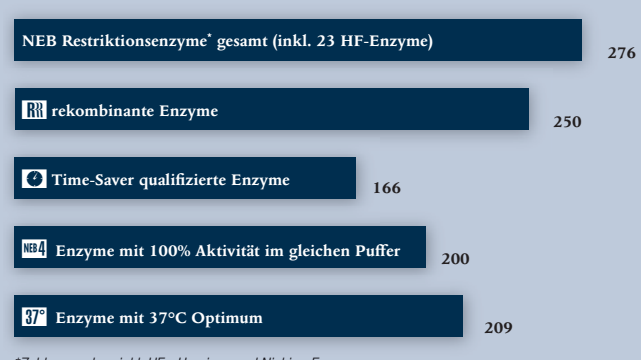

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