

# **ISPP 2012**

*14<sup>th</sup>Internacional Symposium  
on Phototrophic Prokaryotes*

## **Abstract Book**

---



---

August 5 to 10 - Porto, Portugal  
Congress at Porto Palácio Congress Hotel & Spa

**[WWW.IBMC.UP.PT/ISPP2012](http://WWW.IBMC.UP.PT/ISPP2012)**

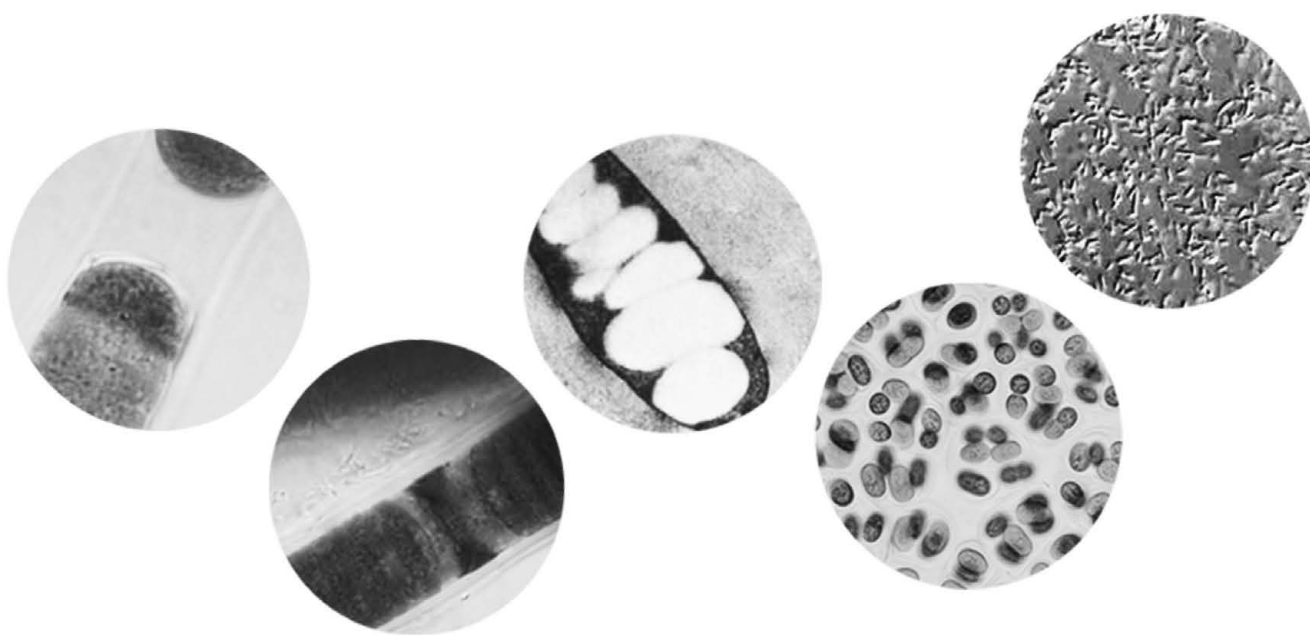
---

# **ISPP 2012**

*14<sup>th</sup>Internacional Symposium  
on Phototrophic Prokaryotes*

## **Abstract Book**

---



---

August 5 to 10 - Porto, Portugal  
Congress at Porto Palácio Congress Hotel & Spa

**[WWW.IBMC.UP.PT/ISPP2012](http://WWW.IBMC.UP.PT/ISPP2012)**



## Welcome address

It's our great pleasure to welcome all participants to the 14th International Symposium on Phototrophic Prokaryotes, in Porto. This symposium aims to cover the current knowledge and the most recent advances of research on phototrophic prokaryotes. The program will focus on the latest scientific achievements in the genetics and physiology of these organisms, also addressing their evolution, ecology, mechanisms of interaction with the environment, and biotechnological applications. In addition, this meeting constitutes an opportunity for a valuable exchange of ideas between scientists with different backgrounds and strengths that can help provide future directions to research works.

In addition to the social events planned, we hope that you are able to get to know and enjoy the beautiful city of Porto. This city is one of the most ancient European cities, classified as World Heritage by UNESCO. Internationally known for its famous Port Wine, the city has other interesting aspects such as landscapes and the historical centre. Besides its welcoming and conservative environment, Porto is also contemporary and artistic. This is shown not only in the streets, architecture, monuments and museums but also in the terraces, restaurants and shopping areas.

The organizing committee is looking forward to a very successful meeting!

Paula Tamagnini  
ISPP 2012 Conference Chair  
IBMC & FCUP  
University of Porto

## Date

5-10 August 2012

## Venue

Porto Palácio Congress Hotel & Spa  
Av. da Boavista 1269, Boavista, 4100-130 Porto

## Event management

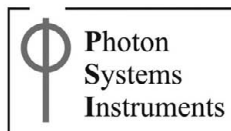
IBMC•INEB Events Management Office  
Antonieta Correia  
Paula Esteves  
Sónia Leitão

## Graphic design

Anabela Nunes

## Sponsors

IBMC - Instituto de Biologia Molecular e Celular  
FCT - Fundação para a Ciência e a Tecnologia  
Reitoria da Universidade do Porto  
Fundação Porto Social  
Alfagene  
Banco Santander Totta  
Photon Systems Instruments  
Fisher Scientific



## Table of contents

<i>Welcome address</i> .....	03
<i>Local Organizing Committee</i> .....	06
<i>International Scientific Committee</i> .....	06
<i>Informations</i> .....	07
<i>Plant of the venue</i> .....	08
<i>Program</i> .....	10
<i>Plenary Sessions</i> .....	15
<i>Oral Communications</i> .....	27
<i>Poster &gt; Session I</i> .....	55
<i>Poster &gt; Session II</i> .....	65
<i>Poster &gt; Session III</i> .....	73
<i>Poster &gt; Session IV</i> .....	121
<i>Poster &gt; Session V</i> .....	143
<i>Participants List</i> .....	160

## Local Organizing Committee:

Paula Tamagnini - pmtamagn@ibmc.up.pt  
 Rosário Martins - mrm@estsp.ipp.pt  
 Sara Pereira - sarap@ibmc.up.pt  
 Vitor Vasconcelos - vvascon@fc.up.pt  
 Joana Gaifem - joana.gaifem@ibmc.up.pt  
 Rita Mota - rita.mota@ibmc.up.pt  
 Ângela Brito - ambrito@ibmc.up.pt

## International Scientific Committee:

**Chair:** Jörg Overman, Germany

### Group I. Reaction centres, antennae, bioenergetics

J. Thomas Beatty, Canada  
 Diana Kirilowsky, France  
 David B. Knaff, USA  
 Min Chen, Australia  
 Kevin Redding, USA  
 Julian Eaton-Rye, New Zealand

### Group II. Metabolism, biosynthesis, physiology

Caroline Harwood, USA  
 Niels Ulrik Frigaard, Denmark  
 Neil Hunter, UK  
 Masahiko Ikeuchi, Japan  
 Oded Beja, Israel  
 Paula M. Tamagnini, Portugal

### Group III. Cell structure and biology, development

Arthur Grossman, USA  
 Bernd Masepohl, Germany  
 Wolfgang Hess, Germany  
 Judith Armitage, UK  
 Cheryl Kerfeld, USA  
 Frédéric Partensky, France

### Group IV. Taxonomy, ecology and evolution

Patrizia Albertano, Italy  
 Ferran Garcia Pichel, USA  
 Akira Hiraishi, Japan  
 Jörg Overmann, Germany  
 Annick Wilmotte, Belgium  
 Vladimir Yurkov, Canadá

## Informations

### Important information

For any information during the meeting, please contact any members of the Conference Services Desk located in the foyer.

### Registration

Registrations will take place in the Conference Services Desk located in the foyer, from 16h00 Sunday (August 5<sup>th</sup>) until 18h00 Monday (August 6<sup>th</sup>).

### Name badgers

For identification and security purposes, participants must wear their name badgers when in the venue. The use of the badge is mandatory for the access to the coffee breaks and lunches.

### Presentation instructions

The plenary sessions should last up to 35-40 minutes followed by 5-10 minutes discussion. Oral presentation should last up to 15 minutes followed by 5 minutes discussion. The chairs are requested to lead the discussion and to ensure that the times are strictly followed.

Speakers presenting in the morning should hand in their presentations in the auditoriums (Sala Porto or Sala Douro) until 8h15 of the presentation day. Speakers presenting in the afternoon sessions, should hand in their presentations during lunch break.

A data-show and PC will be at the presenters' disposal. Technicians will be available to make sure that you have successfully submitted your presentation. You will be requested to provide your presentation on a USB key or CD-rom.

### Poster presentations

Posters should have 1.20m high and 0.90m wide, and will be presented on the designated poster area. Authors are requested to put up their posters during the morning of their poster session and remove it in the end of the next day. Conference staff will be present to provide assistance. Authors should remain next to their poster during the poster session.

**Poster session I** (Tuesday, August 7th, 14h00-16h00): **odd numbers**

[remove on August 8th afternoon]

**Poster session II** (Thursday, August 9th, 14h00-16h00): **even numbers**

[remove on August 10th afternoon]

### Internet access

Wireless internet is provided.

### Lost and found

Lost and found items will be held in the Conference Services Desk located in the foyer.

### Meals and coffee breaks

Coffee breaks will be served in the morning and afternoon.

Lunch will be served at restaurants Madrugá and cervejaria Portobeer.

A limited number of vegetarian, Kosher and Halal meals are available upon previous request.

### Sight Seeing Tour

Buses leave at 17h00 from Porto Palácio Congress Hotel & Spa. The tour will last approximately 2 hours. Buses return to the hotel around 19h00. To register and/or pay for the tour, please refer to the Conference Services Desk located in the foyer. The organization cannot guarantee vacancies for requests made on short notice.

### Dinner party

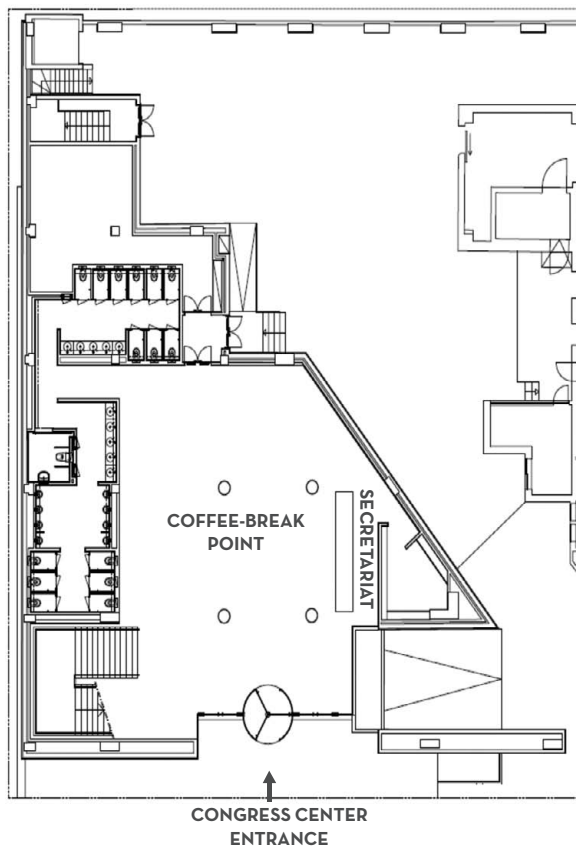
The Dinner party will be held in Círculo Universitário do Porto, and will start at 20h00. To register and/or pay for the dinner, please refer to the Conference Services Desk located in the foyer. The organization cannot guarantee vacancies for requests made on short notice. Vegetarian options are available upon previous request.

**Círculo Universitário do Porto, Rua do Campo Alegre, 877**

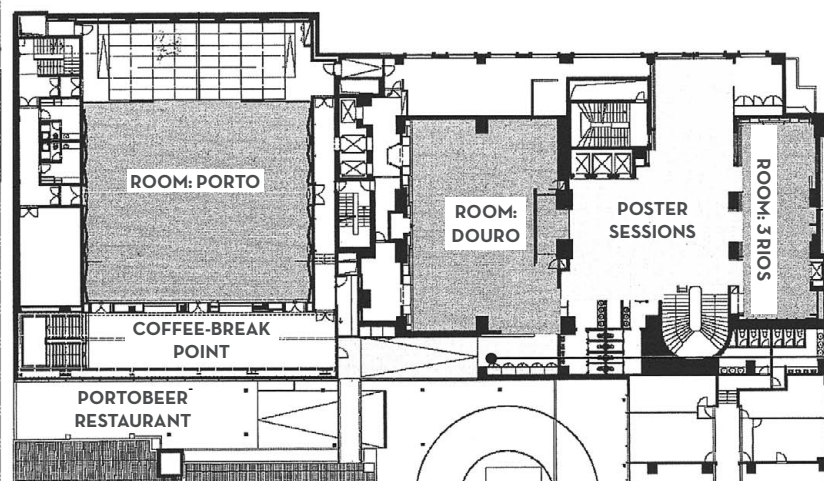


# Plant of the venue

FLOOR 0

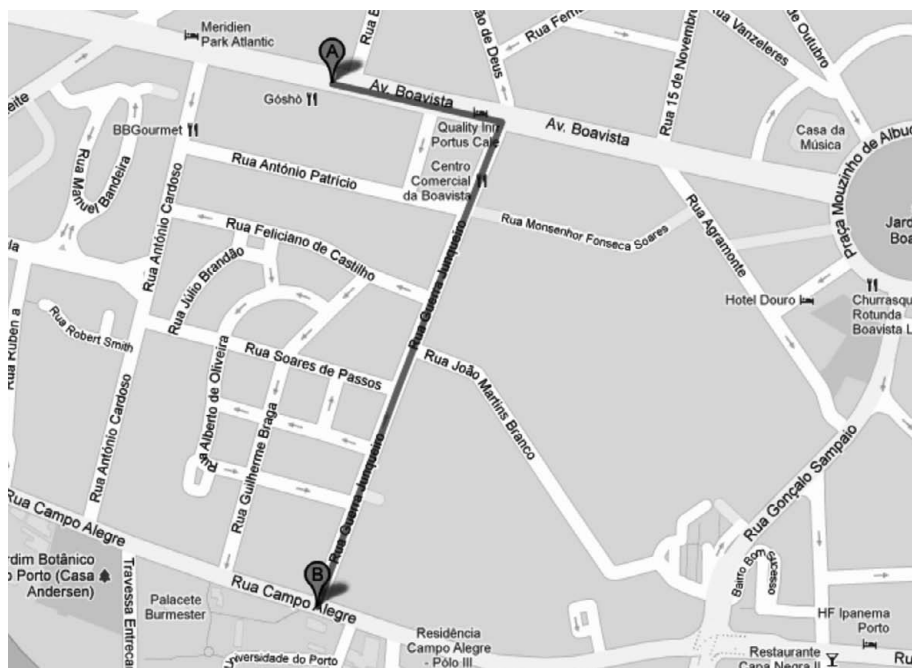


FLOOR 1



LUNCH WILL BE SERVED AT THE HOTEL RESTAURANTS:  
**MADRUGA, FLOOR -1 (CAPACITY: 200)**  
**PORTOBEER, FLOOR 1 (CAPACITY: 100)**

DINNER PARTY WILL BE AT CÍRCULO UNIVERSITÁRIO DO PORTO ON 8TH AUGUST, 20H00  
 REGISTRATION IS REQUIRED



- A** HOTEL PORTO PALÁCIO  
10 MINUTES WALK TO:
- B** CÍRCULO UNIVERSITÁRIO DO PORTO

# Program

## Sunday, August 5<sup>th</sup>

- 16h00** Registration
- 17h30-18h00** Welcome
- 18h00-19h00** **OPENING LECTURE | ROOM: PORTO**  
**PL1: Hugo Scheer:** “Molecular ecology of tetrapyrrole photoreceptors”
- 19h00** Reception

## Monday, August 6<sup>th</sup>

- 8h30-10h00** **SESSION I: PLENARY - PHYLOGENY, TAXONOMY, AND DIVERSITY | ROOM: PORTO**  
**Chair:** Donald Bryant, Pennsylvania State University
- PL2: Debashish Bhattacharya:** “Genomic approaches to understanding the origin of photosynthesis in eukaryotes”
- PL3: Michael Madigan:** “An overview of anoxygenic phototrophs from extreme Environments”
- 10h00-10h30** Coffee break
- 10h30-12h30** **SESSION I: ORAL COMMUNICATIONS (in parallel with session IV) | ROOM: DOURO**  
**Chair:** Johannes Imhoff, Leibniz Institute of Marine Sciences (IFM-GEOMAR); Lucas Stal, Netherlands Institute of Sea Research
- OC1: Michal Koblížek:** “Evolution of photosynthesis in marine *Roseobacters*”
- OC2: Eliška Zapomělová:** “Morphological, molecular and ecological diversity of *Anabaena* spp. (cyanobacteria) without gas vesicles”
- OC3: Vladimir Gorlenko:** “The diversity of anoxygenic phototrophic bacteria in the Buryat and Mongolian thermal springs”
- OC4: Cristiana Moreira:** “Phylogeny and biogeography of the cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*”
- OC5: Jean Huang:** “Characterization of marine and freshwater photosynthetic communities cultured using LEDs”
- 10h30-12h30** **SESSION IV: ORAL COMMUNICATIONS (in parallel with session I) | ROOM: PORTO**  
**Chair:** Jack Meeks, University of California, Davis; Eva-Mari Aro, University of Turku
- OC6: Toivo Kallas:** “A novel, YrdC-like, carbon-stress regulator, active cyclic electron flow at high light intensity, and isoprene production in *Synechococcus* sp. PCC 7002 cyanobacteria”
- OC7: Aaron Setterdahl:** “Identification and characterization of unknown genes in *Rhodobacter sphaeroides*”
- OC8: David Knaff:** “Ferredoxin-dependent cyanobacterial enzymes: mutagenic and biophysical studies”
- OC9: Conrad W. Mullineaux:** “Spatial distribution of thylakoid membrane complexes and partitioning of reducing power in cyanobacteria”
- OC10: Arnaud Taton:** “Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria”
- OC11: Jogadhenu Prakash:** “Hypothetical proteins to unknown cellular mechanisms: with emphasis on a novel transcription factor of heat shock genes in *Synechocystis* sp. PCC6803”

- 12h30-14h00** Lunch
- 14h00-16h00** **SESSION II: ORAL COMMUNICATIONS (in parallel with session V) | ROOM: DOURO**  
**Chair:** Jörg Overmann, Leibniz-Institut DSMZ; Michael Madigan, Southern Illinois University
- OC12: Lucas J. Stal:** "Ecology of coastal cyanobacterial mats"
- OC13: Elke Dittmann:** "Microcystin-binding to proteins is part of the oxidative stress response in *Microcystis aeruginosa*"
- OC14: Shuyi Zhang:** "The TCA cycle in cyanobacteria"
- OC15: Aaron Kaplan:** "The languages spoken in the water body: what determines who is there or the biological role of cyanobacterial toxins"
- OC16: Csaba István Nagy:** "A novel plasmid-borne arsenite and antimonite responsive operon in *Synechocystis* sp. PCC 6803"
- OC17: Sophie Rabouille:** "Fluorescence kinetics in a diazotrophic cyanobacterium: evidence for diurnal state transitions and consistent inhibition during nitrogen fixation"
- 14h00-16h00** **SESSION V: ORAL COMMUNICATIONS (in parallel with session II) | ROOM: PORTO**  
**Chair:** Wolfgang Hess, University of Freiburg; Ann Magnuson, Uppsala University
- OC18: Pia Lindberg:** "Production of isoprenoids in *Synechocystis* PCC 6803"
- OC19: Hans C.P. Matthijs:** "Fast and specific termination of nuisance cyanobacteria with hydrogen peroxide"
- OC20: Hidehiro Sakurai:** "Our challenges for economic photobiological production of H<sub>2</sub> by cyanobacteria"
- OC21: Sara B. Pereira:** "Assembly and export of extracellular polymeric substances (EPS) in cyanobacteria: a phylogenomic approach"
- OC22: Olivier Ploux:** "Secondary metabolism of the cyanobacterium *Oscillatoria* PCC 6506: from genome sequencing to cyanotoxin and metabolite biosyntheses"
- OC23: Peter B. Kós:** "Biotechnological utility of inducible cyanobacterial promoters: biosensors for arsenic and heavy metals"
- 16h00-16h30** Coffee break
- 16h30-18h00** **SESSION II PLENARY: ENVIRONMENT AND ECOLOGY | ROOM: PORTO**  
**Chair:** Cheryl Kerfeld, University of California, Berkeley/ JGI
- PL4: Donald A. Bryant:** "Genomic, metagenomic, and metatranscriptomic analyses of chlorophototrophic microbial mats of hot springs of Yellowstone National Park"
- PL5: David Scanlan:** "Assessing the factors controlling marine picocyanobacterial diversity and abundance"
- Tuesday, August 7<sup>th</sup>**
- 8h30-10h00** **SESSION III PLENARY: PHYSIOLOGY, METABOLISM, SENSING AND SIGNAL TRANSFER | ROOM: PORTO**  
**Chair:** Enrique Flores, Consejo Superior de Investigaciones Científicas
- PL6: Mark Gomelsky:** "Synthetic regulatory modules designed from bacterial photosensors"
- PL7: Gabriele Klug:** "RNA-based regulation in the response of *Rhodobacter sphaeroides* to environmental impacts"
- 10h00-10h30** Coffee break

10h30-12h30

**SESSION III: ORAL COMMUNICATIONS | ROOM: PORTO****Chair:** Peter Lindblad, Uppsala University; Gabriele Klug, University of Giessen**OC24: Ana Valladares:** "Transcriptional activation by NtcA in the absence of consensus NtcA-binding sites in an *Anabaena* sp. PCC 7120 devB promoter"**OC25: Jens Appel:** "Electrochemical characterization of the bidirectional hydrogenase of *Synechocystis* sp. PCC 6803"**OC26: Fevzi Daldal:** "A novel transporter required for biogenesis of cbb3-type cytochrome c oxidase in *Rhodobacter capsulatus*"**OC27: Daniela Ferreira:** "Photosensors and light response/adaptation in *Nostoc punctiforme*"**OC28: Tiago Guerra:** "Altered carbohydrate metabolism in glycogen synthase mutants of *Synechococcus* sp. strain PCC 7002 grown under hypersaline, nitrogen limitation and auto-fermentation conditions"**OC29: Marcus Ludwig:** "Transcription profiling of *Synechococcus* sp. PCC 7002 by RNAseq"

12h30-14h00

Lunch / 12h30-13h30: **Scientific International Committee meeting | ROOM: MINHO/LIMA**

14h00-16h00

**POSTER SESSION | FOYER AND ROOM 3 RIOS**

16h00-16h30

Coffee break

16h30-18h00

**SESSION IV PLENARY: BIOENERGETICS, PROTEOMICS AND GENOMICS | ROOM: PORTO****Chair:** Conrad Mullineaux, Queen Mary University of London**PL8: Wolfgang Hess:** "The regulatory system of cyanobacteria: an RNA perspective"**PL9: Neil Hunter:** "Intracytoplasmic membrane assembly in the purple phototrophic bacterium *Rhodobacter sphaeroides*"**Wednesday, August 8<sup>th</sup>**

8h30-10h00

**SESSION V PLENARY: BIOREMEDIATION, SECONDARY METABOLITES AND OTHER APPLIED ASPECTS | ROOM: PORTO****Chair:** Louis Sherman, Purdue University**PL10: William Gerwick:** Synergizing phylogenetics, natural products drug discovery and biosynthetic investigations of marine cyanobacteria"**PL11: Peter Lindblad:** "Design, engineering, and construction of photosynthetic microbial cell factories for renewable solar fuel production - development of a genetic toolbox"

10h00-10h30

Coffee break

10h30-12h30

**SESSION V PLENARY: BIOREMEDIATION, SECONDARY METABOLITES AND OTHER APPLIED ASPECTS | ROOM: PORTO****Chair:** Peter Nixon, Imperial College London**PL12: Brett Neilan:** "Engineering cyanobacteria and their toxin biosynthesis pathways for unnatural production"**PL13: Roberto De Philippis:** "Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process"

12h00-13h30

Lunch

13h30-15h30

**SESSION V: ORAL COMMUNICATIONS (in parallel with session III) | ROOM: DOURO****Chair:** Ferran Garcia-Pichel, Arizona State University; Vitor Vasconcelos, Faculty of Sciences, University of Porto**OC30: Catarina Pacheco:** "Assembly and characterization of Oxygen Consuming Devices (OCDs): the laccase-based device"**OC31: Kaarina Sivonen:** "Discovery of cyanobacterial peptides by genome mining"**OC32: Teresa Thiel:** "Production of hydrogen by transgenic strains of the cyanobacterium *Anabaena variabilis* expressing hydrogenase genes"**OC33: Toshio Sakamoto:** "Glycosylated mycosporine-like amino acids from the terrestrial cyanobacterium *Nostoc commune*"**OC34: Klaus B. Möllers:** "Carbohydrate loading of cyanobacteria for biofuel fermentation"**OC35: Tanya Soule:** "Accumulation of extracellular carbohydrates in a scytonemin-deficient mutant of *Nostoc punctiforme* exposed to UVA stress"

13h30-15h30

**SESSION III: ORAL COMMUNICATIONS (in parallel with session V) | ROOM: PORTO****Chair:** Martin Hagemann, University of Rostock; David Scanlan, University of Warwick**OC36: Michael L. Summers:** "SigG is an ECF sigma factor required for normal differentiation and stress survival of *Nostoc punctiforme*"**OC37: Samantha J. Bryan:** "Dynamic formation of protein assembly centers in stressed cyanobacteria"**OC38: Martin Hagemann:** "Evolutionary origin of photorespiration: phylogenetic and biochemical studies of core cycle enzymes"**OC39: Shin-Ichi Maeda:** "Nitrite transport activity of the ABC-type cyanate transporter of the cyanobacterium *Synechococcus elongatus* PCC 7942"**OC40: Louis A. Sherman:** "Role of the two hik31 operons in regulating carbohydrate and photosynthetic metabolism in *Synechocystis* sp. PCC 6803"**OC41: Filipe Pinto:** "Mapping and characterization of *Synechocystis* sp. PCC 6803 neutral sites for the integration of synthetic devices"

15h30-16h00

Coffee break

16h00-16h40

**SESSION IV: ORAL COMMUNICATIONS | ROOM: PORTO****Chair:** Brett Neilan, University of New South Wales; Fernando de la Cruz, Universidad de Cantabria**OC42: Min Chen:** "Newly discovered chlorophyll (chlorophyll *f*) and its function in oxygenic photosynthetic organism"**OC43: Igor N. Stadnichuk:** "The terminal emitter LCM (ApcE) as the primary site of non-photochemical quenching of the phycobilisome fluorescence emission by orange carotenoid protein in the cyanobacterium *Synechocystis* sp. PCC 6803"

17h00

Sightseeing tour

20h00

Dinner

**Thursday, August 9<sup>th</sup>****8h30-10h00** **SESSION I PLENARY: PHYLOGENY, TAXONOMY AND DIVERSITY | ROOM: PORTO****Chair:** Aaron Kaplan, The Hebrew University Aaron Kaplan**PL14: Cheryl Kerfeld:** "CyanoGEBA: The cyanobacterial volume of the genomic encyclopedia of Bacteria and Archaea"**PL15: Jörg Overmann:** "Diversity and niche specialization of green sulfur bacteria"**10h00-10h30** Coffee break**10h30-12h30** **SESSION IV: ORAL COMMUNICATIONS | ROOM: PORTO****Chair:** Mark Gomelsky, University of Wyoming; William Gerwick, University of California**OC44: Fernando de la Cruz:** "Mobilization of RSF1010 between *Anabaena* strains illustrates conjugation among cyanobacteria"**OC45: Julian J. Eaton-Rye:** The auxiliary proteins of Photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803"**OC46: Marion Eisenhut:** "Control of the low inorganic carbon induced expression of the flv4-2 operon in *Synechocystis* by the asRNA *As1\_flv4* and the transcriptional regulators *NdhR* and *Sll0822*"**OC47: Andreia F. Verissimo:** "The CcmI subunit of the CcmFHI heme ligation complex is an apocytochrome c chaperone and forms a ternary complex with CcmE and apocytochrome c2"**OC48: Georg Schmetterer:** "The Respiratory terminal oxidases of cyanobacteria"**OC49: Robert A. Niederman:** "Effects of carbonyl-cyanide m-chlorophenylhydrazine on the Induction of Intracytoplasmic membrane assembly in *Rhodobacter sphaeroides*"**12h30-14h00** Lunch**14h00-16h00** **POSTER SESSION | FOYER AND ROOM 3 RIOS****16h00-16h30** Coffee break**16h30-18h00** **SESSION II PLENARY: ENVIRONMENT AND ECOLOGY | ROOM: PORTO****Chair:** Roberto De Philippis, University of Florence**PL16: Jonathan P. Zehr:** "How do we know what we don't know?: Lessons from genomics and metagenomics of uncultivated nitrogen-fixing cyanobacteria"**PL17: Ferran Garcia-Pichel:** "The cyanobacteria from arid soil crusts: a pulsed mode of existence of global proportions"**Friday, August 10<sup>th</sup>****8h30-10h00** **SESSION III PLENARY: PHYSIOLOGY, METABOLISM, SENSING AND SIGNAL TRANSFER | ROOM: PORTO****Chair:** James Golden, University of California**PL18: Jack Meeks:** "Sensing and signaling systems in *Nostoc punctiforme*"**PL19: Eva-Mari Aro:** Alternative electron transfer pathways in cyanobacteria thylakoid membrane"**10h00-10h30** Coffee break



**10h30-12h30****SESSION III: ORAL COMMUNICATIONS | ROOM: PORTO****Chair:** Himadri Pakrasi, Washington University; Toivo Kallas, University of Wisconsin Oshkosh**OC50: Zahra Choolei:** "Rhodobacter capsulatus AmtY is functional when expressed in *Escherchia coli*"**OC51: Nir Keren:** "Iron and manganese in cyanobacteria: a story of co-regulation"**OC52: Vicente Mariscal:** "Proteins joining the cells in the filaments of *Anabaena* sp. PCC 7120"**OC53: Niels-Ulrik Frigaard:** "Mechanisms and evolution of growth on sulfur compounds in phototrophic bacteria"**OC54: Karin Stensjö:** "The role of Dps (DNA-binding proteins from starved cells) -like proteins in the adaptations to environmental perturbations in cyanobacteria"**OC55: Susan Golden:** "Impairment of O-antigen production confers resistance to grazing in a model cyanobacterium-amoeba predator-prey system"**12h30-14h00**

Lunch

**14h00-16h00****SESSION IV PLENARY: BIOENERGETICS, PROTEOMICS AND GENOMICS | ROOM: PORTO****Chair:** Debashish Bhattacharya, Rutgers University**PL20: Himadri Pakrasi:** "A day and a night in the life of a unicellular diazotrophic cyanobacterium"**PL21: Peter Nixon:** "Assembling and maintaining the Photosystem II complex in cyanobacteria"**16h00-16h30**

Coffee break

**16h30-17h00****CLOSING LECTURE | ROOM: PORTO****PL22: Vitor Vasconcelos:** "Cyanobacteria and eukaryotes: do they dialogue or not?"**17h00-18h00**

Closing session

# PLENARY SESSIONS





## PLENARY SESSIONS

### PL1: Molecular Ecology of Tetrapyrrole Photoreceptors

Hugo Scheer<sup>1</sup> and Kai-Hong Zhao<sup>2</sup>

<sup>1</sup>Dept. Biologie 1 – Botanik, Universität München, München, Germany; <sup>2</sup>State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China

Cyclic tetrapyrroles, the chlorophylls (Chl), serve as light-harvesting pigments and redox carriers, linear tetrapyrroles serve as light-harvesting and photosensory pigments. In both cases, the biophysical (optical spectra, excited state dynamics) and biochemical properties (photochemistry, redox potentials) of the chromophores in the native pigment-proteins differ markedly from those of the respective free pigments, thereby adapting them to particular functions. Molecular mechanisms for this “molecular ecology” will be discussed with two pertinent examples, focusing on the optical properties.

Chl *a* is the most abundant pigment of oxygenic photosynthesis. For a long time, it was the only Chl functional in reaction centers of both photosystems. Its intense absorption and long-lived excited states have evolved by structural modifications of the biosynthetic precursor, protoporphyrin. A drawback are the narrow bands that cover only the edges of the visible spectrum. Between these bands of Chl *a*, numerous additional pigments serve for increasing light-harvesting. Oxygenic photosynthesis can, however, also extend into near IR spectral region, exploiting niches where light <700 nm is absent due to shading by Chl *a* containing phototrophs. Two strategies are known: excitonic coupling, and a modification of Chl *a* by peripheral CHO-groups. The first red-shifted pigment, Chl *d*, is also functional in RC, thereby extending the energetic limits for water splitting<sup>1</sup>. Cyanobacteria containing the novel Chl *f* show even larger red-shifts<sup>2</sup>.

Biliproteins are light-harvesting pigments and sensory photoreceptors of cyanobacteria. A wide spectral coverage is achieved by a combination of non-covalent interactions with the apoproteins; and structural modifications after post-translational chromophore attachment. The best-studied chromophore is phycocyanobilin. In the native biliproteins, its absorption is increased 5-10fold, excited state lifetimes are increased by up to four orders of magnitude, and absorption maxima can vary by more than 100 nm; these changes occur in a hierarchical order<sup>3</sup>. Structural modifications allowing even larger spectral coverage relate to a double-bond shift occurring during covalent attachment<sup>4</sup>. The resulting PVB chromophore is found in phyco-erythrocyanin<sup>5</sup> and certain cyanobacteriochromes, CBCR<sup>6</sup>. The “two-Cys” subclass of CBCR contains chromophores absorbing even below 500 nm and into the near UV. Here, a second thioether bond is formed that interrupts conjugation of the tetrapyrrole; it is maintained in some CBCRs after the photoreaction, and cleaved reversibly in others<sup>7</sup>. Only few of these CBCRs have been characterized functionally, but their spread from 300 – 730 nm, red- or blue-shifts upon photoisomerization, and the wide range of kinetics of the photoconversion and dark reversion would potentially allow cyanobacteria exploiting, in unprecedented detail, the light environment and, thereby, the determining abiotic and biotic factors.

[1] Swingley, W. D.; Chen, M.; Cheung, P. C.; Conrad, A. L.; Dejesa, L. C.; Hao, J.; Honchak, B. M.; Karbach, L. E.; Kurdoglu, A.; Lahiri, S.; Mastrian, S. D.; Miyashita, H.; Page, L.; Ramakrishna, P.; Satoh, S.; Sattley, W. M.; Shimada, Y.; Taylor, H. L.; Tomo, T.; Tsuchiya, T.; Wang, Z. T.; Raymond, J.; Mimuro, M.; Blankenship, R. E.; Touchman, J. W. *Proc Natl Acad Sci U S A* 2008, 105, 2005-2010; [2] Chen, M.; Schliep, M.; Willows, R. D.; Cai, Z.-L.; Neilan, B. A.; Scheer, H. *Science* 2010, 329, 1318-1319 [3] Kupka, M.; Scheer, H. *Biochim. Biophys. Acta* 2008, 1777, 94-103; [4] Scheer, H.; Zhao, K.-H. *Mol. Microbiol.* 2008, 68, 263-276; [5] Bryant, D. A. *J. Gen. Microbiol.* 1982, 128, 835-844; [6] Ishizuka, T.; Kamiya, A.; Suzuki, H.; Narikawa, R.; Noguchi, T.; Kohchi, T.; Inomata, K.; Ikeuchi, M. *Biochemistry* 2011, 50, 953-961; [7] Rockwell, N. C.; Martin, S. S.; Feoktistova, K.; Lagarias, J. C. *Proc. Natl. Acad. Sci. USA* 2011, 108, 11854-11859

### PL2: Genomic Approaches to Understanding the Origin of Photosynthesis in Eukaryotes

Debashish Bhattacharya

Department of Ecology, Evolution and Natural Resources and Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, New Jersey, 08901, USA

Much of our understanding of the natural world has come from experiments done on model species manipulated under controlled laboratory conditions. The advent of modern high-throughput genomics and bioinformatics allows researchers to explore genetic diversity in natural systems, opening the way for exploring organism biology *in situ*. Here I will discuss work from our lab on the genomes of key algal groups that help us understand plastid and eukaryote evolution. One focus will be recently sequenced genome of the glaucophyte *Cyanophora paradoxa* that allowed us to unite the Plantae (also known as Archaeplastida) as a monophyletic group<sup>1</sup>. I will also discuss how we have developed single-cell genomic methods to interrogate DNA associated with individual cells captured in nature<sup>2</sup>. We target unicellular eukaryotes in their natural environment and generate draft genome assemblies to discover novel biodiversity, to reconstruct their biotic interactions, and to generate *de novo* assemblies of associated symbiont and pathogen genomes<sup>3</sup>.

[1] Price, D.C., C.X. Chan, H.S. Yoon, E.C. Yang, H. Qiu, A.P.M. Weber, R. Schwacke, J. Gross, N.A. Blouin, C. Lane, A. Reyes-Prieto, D.G. Durnford, J.A.D. Neilson, B.F. Lang, G. Burger, J.M. Steiner, W. Löffelhardt, J.E. Meuser, M.C. Posewitz, S. Ball, M.C. Arias, B. Henrissat, P.M. Coutinho, S.A. Rensing, A. Symeonidi, H. Doddapaneni, B.R. Green, V.D. Rajah, J. Boore, and D. Bhattacharya. 2012. *Cyanophora paradoxa* genome elucidates origin of photosynthesis in algae and plants. *Science* 335: 843-847; [2] Yoon, H.S., D.C. Price, R. Stepanauskas, V.D. Rajah, M.E. Sieracki, W.H. Wilson, E.C. Yang, S. Duffy, and D. Bhattacharya. 2011. Single cell genomics reveals trophic interactions and evolutionary history of uncultured protists. *Science* 332: 714-717; [3] Bhattacharya, D., D.C. Price, H.S. Yoon, E.C. Yang, N.J. Poulton, R.A. Andersen, and S.P. Das. 2012. Single cell genome analysis supports a link between phagotrophy and primary plastid endosymbiosis. *Nature Sci. Rep.* 2: 356.

### PL3: An Overview of Anoxygenic Phototrophs from Extreme Environments

**Michael T. Madigan**

Department of Microbiology, Southern Illinois University, Carbondale, Illinois 62901 USA

Anoxic extreme environments receiving some illumination are potential habitats for anoxygenic phototrophic bacteria. Over the past 40 years several taxa of purple and green bacteria and heliobacteria have been isolated from extreme environments and used to probe the environmental limits to photosynthesis<sup>1</sup>. The best-studied extremophilic anoxygenic phototrophs have been thermophilic species. The filamentous green nonsulfur bacterium *Chloroflexus aurantiacus* was isolated from alkaline hot springs worldwide in the early 1970s. This organism displayed an astonishing array of novel characteristics including its phylogeny, mechanism of autotrophy, and photosynthetic properties. In the early 1980s, the thermophilic purple sulfur bacterium *Thermochromatium tepidum* was isolated from neutral sulfidic Yellowstone hot springs. Besides its thermophilic phenotype, *Tch. tepidum* was the first of now several examples of purple bacteria whose LH1 antenna absorbs substantially to the red of its reaction center. In the late 1980s, the green bacterium *Chlorobaculum tepidum* was isolated from acidic sulfidic New Zealand hot springs. Because of its rapid growth, capacity for genetic manipulation, and pioneering genomic sequence, *Cba. tepidum* revolutionized the study of green bacteria; indeed, most laboratory studies of green sulfur bacteria today employ this species. More recently, genomic insights from the thermophile *Heliobacterium modesticaldum* have revealed the lifestyle of a "minimalist phototroph", including the organism's streamlined photocomplexes and lack of autotrophy. New anoxygenic phototrophs from Antarctic lakes and soda lakes offer unique opportunities for peering at photosynthesis in these extreme environments, as well. For example, studies of the psychrophile *Rhodospirillum rubrum antarcticum* could reveal key adaptations necessary for photosynthesis in the cold, and the discovery of several new soda lake heliobacteria<sup>2</sup> suggest a previously unrecognized ecological role for these phototrophs in highly alkaline environments. Complete genome sequences of *Rfx. antarcticum* and the alkaliphilic *Heliorestis convoluta* are in hand and should greatly assist in these efforts. For unknown reasons, while soda lakes contain a diverse assemblage of purple sulfur bacteria and heliobacteria, purple nonsulfur bacteria are rare in these environments and green sulfur bacteria appear to be absent altogether. Acidic environments have not been well surveyed for anoxygenic phototrophs and to date, only three species of purple nonsulfur bacteria can be grown below pH 5. Examples of phototrophic diversity in some unusual extreme environments will also be presented, as well as highlights of what extremophilic anoxygenic phototrophic bacteria have taught us about photosynthesis.

[1] Madigan M.T. (2003). Anoxygenic phototrophs from extreme environments. *Photosyn. Res.* 76: 157–171; [2] Asao M, and Madigan M.T. (2010). Taxonomy, phylogeny, and ecology of heliobacteria. *Photosyn. Res.* 104: 103–111

### PL4: Genomic, Metagenomic, and Metatranscriptomic Analyses of Chlorophototrophic Microbial Mats of Hot Springs of Yellowstone National Park

**Donald A. Bryant<sup>1,2</sup>, Zhenfeng Liu<sup>1</sup>, Amaya M. Garcia Costas<sup>1</sup>, Yusuke Tsukatani<sup>1</sup>, Marcus Ludwig<sup>1</sup>, Christian G. Klatt<sup>3</sup>, Jason M. Wood<sup>3</sup>, Niels-Ulrik Frigaard<sup>4</sup>, Sheila I. Jensen<sup>4</sup>, Michael Kühl<sup>4</sup>, Douglas B. Rusch<sup>5</sup>, and David M. Ward<sup>3</sup>**

<sup>1</sup>Dept. of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA; <sup>2</sup>Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717 USA; <sup>3</sup>Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717 USA; <sup>4</sup>Marine Biology Section, Department of Biology, University of Copenhagen, Copenhagen, Denmark  
<sup>5</sup>Department of Genomics and Bioinformatics, Indiana University, Bloomington, IL 47405 USA

The chlorophototrophic microbial mats of effluent channels at ~60°C of Mushroom Spring, an alkaline siliceous hot spring in the Lower Geyser Basin of Yellowstone National Park, have been studied by genomic, metagenomic and metatranscriptomic methods. Complete genomic sequences for several representative isolates and analyses of assembled shotgun metagenomic sequences identified seven major chlorophototrophic populations. The data permitted the identification and characterization of predominant community members and their physiological potentials [1]. Three novel chlorophototrophic populations were identified: "*Candidatus Chloracidobacterium thermophilum*" (*Acidobacteria*), "*Candidatus Thermochlorobacter aerophilum*" (*Chlorobi*), and an *Anaerolineae*-like chlorophototroph, which is only distantly related to members of the *Chloroflexales* (*Chloroflexi*). The mats additionally contain populations related to *Synechococcus* spp. (*Cyanobacteria*) as well as *Roseiflexus* spp. and *Chloroflexus* spp. (*Chloroflexi*). The genomic and annotated metagenomic data served as the reference dataset for transcription profiling using a next-generation sequencing platform (SOLiD) [2]. RNA samples were collected at 1-h intervals throughout a full diel cycle. Transcription profiles were generated for each of the major chlorophototrophs in the mats, and the data were analyzed by k-means clustering to determine the major patterns of transcription for each as a function of the diel cycle. The results from these transcription profiles will be discussed.

The complete genome of "*Ca. C. thermophilum*" has been determined. The genome is encoded on two chromosomes of 2.6 and 1.1 Mb, and both encode essential genes. These data and cultivation studies verify that this organism is an aerobic chlorophototroph [3]. The type-1 homodimeric reaction centers of this bacterium are oxygen tolerant, contain a unique carotenoid-binding protein, and additionally contain three different types of chlorophylls: BChl *a*, Chl *a*, and Zn-BChl *a'* [4]. This bacterium has a complete aerobic respiratory electron transport chain composed of Type-1 NADH dehydrogenase, alternative Complex III, and cytochrome *c* oxidase. "*Ca. T. aerophilum*" defines a new family-level lineage within the phylum *Chlorobi*. Unlike green sulfur bacteria, this still uncultivated bacterium cannot oxidize sulfur compounds and cannot fix nitrogen [5]. ATP-citrate lyase is missing, and the organism has an electron transport chain that is nearly identical to that of a known aerobe, "*Ca. C. thermophilum*." However, unlike "*Ca. C. thermophilum*," which produces chlorosomes containing BChl *c*, "*Ca. T. aerophilum*" lacks the *bchU* gene and therefore produces chlorosomes containing BChl *d*.

[1] Klatt et al. 2011. ISME J 5,1262-1278; [2] Liu et al. 2011. ISME J 5,1279-1290; [3] Garcia Costas et al., 2012. *Env Microbiol* 14, 177-190; [4] Tsukatani et al., 2012. *J Biol Chem* 287, 5720-5732; [5] Liu et al. 2012. ISME J, in press;

## PL5: Assessing the factors controlling marine picocyanobacterial diversity and abundance

**D. J. Scanlan<sup>1</sup>, F. Pitt<sup>1</sup>, M. Ostrowski<sup>1, 2</sup>, S. Mazard<sup>1, 2</sup>, C. Grob<sup>1</sup>, L. Jardillier<sup>1, 3</sup>, M. Hartmann<sup>4</sup>, M. V. Zubkov<sup>4</sup>**

<sup>1</sup>School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK; <sup>2</sup>Biomolecular Frontiers Research Centre, Macquarie University, Australia

<sup>3</sup>Université Paris-Sud 11, Orsay, France; <sup>4</sup>National Oceanography Centre, Southampton, UK

Marine picocyanobacteria of the genera *Synechococcus* and *Prochlorococcus* are numerically dominant in vast tracts of the ocean. Using a combination of  $\text{NaH}^{14}\text{CO}_3$  radiotracer and flow cytometric sorting studies we can now evaluate the importance of each genera in marine  $\text{CO}_2$  fixation [1]. Although a minor component numerically, pico-sized photosynthetic eukaryotes i.e. <2-3  $\mu\text{m}$  in diameter, are also important marine primary producers, being responsible for up to 40% of total primary production at low latitudes such as the North-Eastern tropical Atlantic [1]. These organisms encompass many uncultured lineages of the classes Prymnesiophyceae and Chrysiophyceae, some of which are likely critical for controlling picocyanobacterial numbers *in situ*, given recent evidence showing the importance of mixotrophy by plastidic protists in oligotrophic gyre ecosystems [2]. Biotic control of natural picocyanobacterial populations likely also includes viral lysis. We present new evidence that also suggests interactions between the grazing and viral lysis process.

As well as biotic control of cell abundance, natural picocyanobacterial populations are also structured genetically by abiotic factors such as temperature, nutrient availability and light intensity [3]. Specifically for *Synechococcus*, we have developed a high resolution phylogenetic framework to fine tune the geographical partitioning of this genus *in situ* [4]. In concert with in-depth genomic and metagenomic studies, we aim to uncover the specific adaptation mechanisms of the numerous *Synechococcus* phylotypes observed, that will help explain the successful colonization of this genus throughout the marine environment.

[1] Jardillier et al., (2010) ISME J 4: 1180-1192; [2] Hartmann et al., (2012) PNAS 109: 5756-5760; [3] Scanlan et al., (2009) MMBR 73: 249-299; [4] Mazard et al., (2012) Env Micro 14: 372-386

## PL6: Synthetic regulatory modules designed from bacterial photosensors

**Mark Gomelsky**

Department of Molecular Biology, University of Wyoming

Phototrophic bacteria use diverse photosensory proteins to adjust photosynthetic activity, to avoid photooxidative damage, for phototactic responses and for transitioning between the motile and sessile lifestyles. An overview of bacterial photoreceptors will be presented and examples of light-regulated physiology and behavior will be discussed. The focus of the presentation will be on utilizing bacterial photosensory proteins as tools for synthetic biology. Light-activated systems are attractive because light can control biological processes with high spatiotemporal resolution. Synthetic light-activated regulatory modules can be designed and delivered as genes into microbial and animal cells to control biological properties *in vivo*. For applications in mammalian models, bacteriophytochromes, the photoreceptors that absorb far-red/near-infrared light are of particular interest because light in the range of 670-800 nm penetrates deep into mammalian tissues, and because their chromophore, biliverdin IX $\alpha$ , is naturally produced in the heme degradation pathway. Two bacteriophytochrome-based synthetic modules for light-activated control of gene expression and protein activities will be discussed. Both are based on the bacteriophytochrome BphG1 from the anoxygenic facultatively phototrophic bacterium *Rhodobacter sphaeroides*. The first system involves a light-regulated gene expression using the bacterial second messenger c-di-GMP as intermediate. The second system involves engineered chimeric bacteriophytochromes with desired output activities that respond to near-infrared light. These photoactivated systems could offer unprecedented insights into diverse biological processes as well as lead to new disease treatments.

## PL7: RNA-based regulation in the response of *Rhodobacter sphaeroides* to environmental impacts

**Gabriele Klug**

Institute of Microbiology and Molecular Biology, University of Giessen, Heinrich-Buff-Ring 26-32, D 35392 Giessen, Germany

The formation of photosynthetic complexes is regulated by oxygen tension and light in facultative photosynthetic bacteria. For many years members of the genus *Rhodobacter* have served as model systems to study regulation of genes for photosynthesis, nitrogen fixation, carbon dioxide fixation and oxidative stress responses. Many protein regulators have been identified that build up complex regulatory networks to allow the adaptation to changing environmental conditions. Over the last decade the important role of regulatory small RNAs (sRNAs) in bacterial responses emerged. By RNAseq we have identified several sRNAs, which play a role in regulation of photosynthesis gene expression and/or diverse stress responses. Some of these sRNAs have been investigated in detail and their role in regulatory networks and their mechanisms of action will be presented.

[1] Berghoff, B.A., Glaeser, J., Sharma, C., Vogel, J., Klug, G. (2009) Photooxidative stress induced and abundant small RNAs in *Rhodobacter sphaeroides*. Mol. Microbiol. 74: 1497-1512; [2] Berghoff, B.A., Glaeser, J., Nuss, A.N., Zobawa, M., Lottspeich, F., Klug, G. (2011) Aerobic photosynthesis and photooxidative stress: A particular challenge for *Roseobacter*. Environm. Microbiol. 13: 775-792; [3] Berghoff, B.A., Glaeser, J., Sharma, C.M., Zobawa, M., Lottspeich, F., Vogel, J., Klug, G. (2011) Contribution of Hfq to photooxidative stress resistance and global regulation in *Rhodobacter sphaeroides*. Mol. Microbiol. 80: 1479-1495

## PL8: The Regulatory System of Cyanobacteria: An RNA Perspective

**Wolfgang R. Hess, Jens Georg, Matthias Kopf, Björn Voß**

University of Freiburg, Faculty of Biology, 79104 Freiburg, Germany;

The ability for adaptation to vastly different environmental conditions suggests the existence of sophisticated regulatory mechanisms in cyanobacteria. Therefore, regulatory proteins can be expected to interplay with various types of regulatory RNA molecules within the different signal transduction pathways and stress responses. However, comprehensive data on the architecture and composition of the cyanobacterial transcriptome have been lacking for a long time. More recently, we have experimentally characterized the transcriptomes of the unicellular model organism *Synechocystis* sp. PCC6803 and of the nitrogen-fixing filamentous *Anabaena* sp. PCC7120, identifying more than 3,000 and 10,000 active promoters, respectively [1-3].

Our results suggest a surprisingly complex transcriptome architecture: Only about 35% of all promoters drive the expression of protein-coding genes and operons, whereas the remaining 65% transcribe various types of non-coding RNAs (ncRNAs). The functional characterization of this class of potentially regulatory RNA molecules is a constant challenge. To enable a comparative approach, we now have sequenced the transcriptomes of *Synechocystis* sp. PCC6803 and of *Synechocystis* sp. PCC6714, a close relative, under 10 different conditions. This double-comparative approach (different conditions and two different organisms) identified several previously uncharacterized and strongly regulated transcripts, yielded a number of conserved genes, potentially trans-acting ncRNAs, as well as antisense transcripts with identical regulation and tripled the number of known promoters for *Synechocystis*. The latter result is due to the fact that many transcriptional start sites are specific for one or a few conditions only.

We show some of the ncRNAs to fulfill distinct roles in the control of stress responses and the optimization of photosynthesis. Modelling the molecular and functional interactions of cyanobacterial ncRNAs globally suggests an array of distinct molecular mechanisms that need to be integrated into the fabric of the intracellular regulatory network. This work is relevant for the establishment of long-time producer strains for 3rd generation biofuels and for understanding the adaptation potential of cyanobacterial primary producers in a changing world [4].

[1] Georg J., Voß B., Scholz I., Mitschke J., Wilde A., Hess W.R. (2009): Evidence for a major role of antisense RNAs in cyanobacterial gene regulation. *Mol. Sys. Biol.* 5, 305; [2] Mitschke J., Georg J., Scholz I., Sharma C., Dienst, D., Bantscheff J., Steglich C., Voß B., Wilde, A., Vogel J., Hess W.R. (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC 6803. *Proc. Natl. Acad. Sci. USA* 108, 2124-2129; [3] Mitschke J., Vioque A., Haas F., Hess W.R., Muro-Pastor A.M. (2011) Dynamics of transcriptional start site selection during nitrogen stress-induced cell differentiation in *Anabaena* sp. PCC7120. *Proc. Natl. Acad. Sci. USA* 108, 20130-20135; [4] Hess W.R. (2011) Cyanobacterial genomics for ecology and biotechnology. *Curr. Opin. Microbiol.* 14, 608-614.

## PL9: Intracytoplasmic membrane assembly in the purple phototrophic bacterium *Rhodobacter sphaeroides*

**C. Neil Hunter**

Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK

The purple phototrophic bacteria increase the surface area for absorbing and utilizing solar energy by synthesizing an extensive system of intracytoplasmic membranes (ICM). In the purple phototrophic bacterium *Rhodobacter (Rba.) sphaeroides* the ICM system comprises hundreds of rounded invaginations, approximately 50 nm in diameter. Cryo-electron tomography of *Rba. sphaeroides* shows that some of the ICM vesicles are connected to the cytoplasmic membrane by a narrow neck, whereas other vesicles are free-living in the cytoplasm, both singly and attached to one another. Such 'free-living' ICM vesicles, which possess all the machinery for converting light energy into ATP, can be regarded as bacterial membrane organelles. In addition to ICM vesicles the tomographic analysis indentified indentations of the cytoplasmic membrane, which could be isolated as gently curved disc-shaped membranes, termed UPB, when extracts of disrupted cells were fractionated on sucrose density gradients. Pulse-chase radiolabeling studies showed that these membrane discs are the biosynthetic precursor of mature, fully invaginated ICM vesicles, and are likely to originate from sites in the cytoplasmic membrane where the process of membrane invagination is initiated. Electron, atomic force and fluorescence microscopy analysis of these UPB precursor membranes showed that dimeric reaction centre light-harvesting 1-PufX core complexes are present; however, light-harvesting LH2 complexes are present in reduced amounts, and are only partly connected for energy transfer to the core complexes. Thus, solar energy harvesting is not fully established in this precursor membrane.

In order to characterize the UPB precursor and mature intracytoplasmic membranes in more detail qualitative and quantitative proteomic analyses were carried out, using multiple extraction techniques prior to protein identification by mass spectrometry. 387 proteins were identified, representing 9% of the total theoretical proteome of *Rba. sphaeroides*. 43 proteins were found only in the ICM fraction and 236 were unique to the precursor UPB fraction; 107 proteins were determined to be common to both photosynthetic membrane types. The relatively small proteome of the ICM fraction reflects its specialised role within the cell, the conversion of light into chemical energy. The significantly more complex proteome of the UPB precursor membrane, while including photosynthetic reaction center and light-harvesting complexes, is closer to that of undifferentiated cytoplasmic membrane. This comparison between precursor and mature photosynthetic membranes was enhanced by using metabolic stable isotope labelling with <sup>15</sup>N to quantify the relative abundance of proteins in ICM and UPB membrane fractions. We provide further evidence to support the developmental precursor-to-product relationship existing between UPB and ICM in which energy-transducing proteins are systematically enriched in the growing ICM to create a specialised bacterial organelle for converting light into ATP.

## PL10: Synergizing Phylogenetics, Natural Products Drug Discovery and Biosynthetic Investigations of Marine Cyanobacteria

Niclas Engene<sup>1</sup>, Rashel V. Grindberg<sup>1</sup>, Tara Byrum<sup>1</sup>, Emily Monroe<sup>1</sup>, Adam Jones<sup>1</sup>, David H. Sherman<sup>2</sup>, Janet L. Smith<sup>2</sup>, Fred Valeriote<sup>3</sup>, Lena Gerwick<sup>1</sup>, and William H. Gerwick<sup>1,4</sup>

<sup>1</sup> Scripps Institution of Oceanography, University of California San Diego, La Jolla, California, USA; <sup>2</sup> Life Sciences Institute and Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan, USA; <sup>3</sup> Henry Ford Health System, Department of Internal Medicine Josephine Ford Cancer Center, 440 Burroughs, Room 415, Detroit, Michigan, USA; <sup>4</sup> Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, La Jolla, California, USA

Cyanobacteria are an exceptionally rich source of biologically active secondary metabolites, especially those with activities relevant to cancer, neurodegenerative diseases, infectious diseases, and inflammation. An early discovery of our laboratory was from a benthic filamentous marine cyanobacterium, identified as *Lyngbya majuscula*, collected from Curaçao in the Southern Caribbean. Following a very potent cancer cell toxicity associated with the extract, we isolated and defined a novel lipid metabolite given the common name 'curacin A' [1]. Subsequently, we have engaged in detailed investigations of the chemistry of this new structure class, its pharmacological activities, and the pathway for its biosynthesis. These later studies have been especially intriguing and led to several discoveries of new biosynthetic mechanisms in Nature [2]. In a second project, the Papua New Guinea marine cyanobacterium, *Lyngbya bouillonii*, was studied in depth for its anticancer natural products, in particular the apratoxins, as well as their pathways of biosyntheses [3]. We were motivated to undertake genome sequencing of these two species, which has deeply revealed their metabolic and life history characteristics [4]. Moreover, it became increasingly clear that neither of these species of 'Lyngbya' were closely related to the type specimen of this genus, thus a multidisciplinary approach to determine their true phylogenetic relationships to other marine cyanobacteria was undertaken (traditional morphology, habitat, ultrastructure, chemistry, phylogenetics and genomics). As a result of these studies, we found that these two species were members of an undescribed marine cyanobacterial genus which we have recently named *Moorea* gen. nov. [5].

[1] Gerwick, W.H., Proteau, P.J., Nagle, D.G., Hamel, E., Blokhin, A., and Slate, D. J. Org. Chem. 1994, 59, 1243-1245; [2] Gu, L., Wang, B., Kulkarni, A., Geders, T. W., Grindberg, R. V., Gerwick, L., Håkansson, K., Wipf, P., Smith, J. L., Gerwick, W. H. and Sherman, D. H. Nature 2009, 459, 731-735; [3] Grindberg, R.V.; Ishoey, T.; Brinza, D.; Esquenazi, E.; Coates, R.C.; Liu, W.; Gerwick, L.; Dorrestein, P.C.; Pevzner, P.; Lasken, R.; Gerwick, W.H. PLoS One 2011, 6, e18565; [4] Jones, A.C.; Monroe, E.A.; Podell, S.; Hess, W.R.; Klages, S.; Esquenazi, E.; Niessen, S.; Hoover, H.; Rothmann, M.; Lasken, R.S.; Yates, J.R., III; Reinhardt, R.; Kube, M.; Burkart, M.D.; Allen, E.E.; Dorrestein, P.C.; Gerwick, W.H.; Gerwick, L. PNAS 2011, 108, 8815-8820; [5] Engene, N.; Kaštrovský, J.; Rottacker, E.C.; Byrum, T.; Chol, H.; Ellisman, M.H.; Gerwick, W.H. IJSEM 2012 (in press).

## PL11: Design, Engineering, and Construction of Photosynthetic Microbial Cell Factories for Renewable Solar Fuel Production - Development of a Genetic Toolbox

**Peter Lindblad**

Photochemistry and Molecular Science, Department of Chemistry – Ångström Laboratory, Uppsala University, Box 23, SE-751 20 Uppsala, Sweden. Peter.Lindblad@kemi.uu.se

There is an urgent need to develop sustainable solutions to convert solar energy into energy carriers used in the society. In addition to solar cells generating electricity, there are several options to generate solar fuels.

Native and engineered cyanobacteria have been as model systems to examine, demonstrate, and develop photobiological H<sub>2</sub> production. More recently, the production of carbon-containing solar fuels like ethanol, butanol, and isoprene have been demonstrated, see [1]. We have initiated a development of a standardized genetic toolbox, using a synthetic biology approach, to custom-design, engineer and construct cyanobacteria to produce a desired product [1, 2]. One bottleneck is a controlled transcription of introduced genetic constructs. I will present and discuss recent progress in the design, construction and use of artificial genetic elements for the regulation of transcription in cyanobacteria. In addition, I will introduce the concept of light regulated promoters [3], and discuss the use of the CRISPR system as a potential bacterial genetic defence mechanism to achieve robust, cyanobacterial cultures in larger production units [4].

[1] Lindblad, P., Lindberg, P., Oliveira, P., Stensjö, K. and Heidorn, T. 2012. Design, Engineering, and Construction of Photosynthetic Microbial Cell Factories for Renewable Solar Fuel Production. *AMBIO* 41 (Suppl 2): 163-168; [2] Heidorn, T., Camsund, D., Huang, H.-H., Lindberg, P., Oliveira, P., Stensjö, K. and Lindblad, P. 2011. Synthetic Biology in Cyanobacteria: Engineering and Analyzing Novel Functions. *Methods in Enzymology* 497: 540-579; [3] Camsund, D., Lindblad, P. and Jaramillo, A. 2011. Genetically engineered light sensors for control of bacterial gene expression. *Biotechnology Journal* 6 (7): 826-836; [4] Agervald, Å., Camsund, D., Stensjö, K. and Lindblad, P. 2012. CRISPR in the extended *hyp*-operon of the cyanobacterium *Nostoc* sp. strain PCC 7120, characteristics and putative function(s). *International Journal of Hydrogen Energy* 37 (10): 8828-8833.



## **PL12: Engineering cyanobacteria and their toxin biosynthesis pathways for unnatural production**

**Brett A. Neilan**

School of Biotechnology and Biomolecular Sciences, The University of New South Wales (<http://neilan.babs.unsw.edu.au/>)

The past ten years has witnessed major advances in our understanding of natural product biosynthesis, including the genetic basis for toxin production by a number of groups of bacteria and fungi. Cyanobacteria produce an unparalleled array of bioactive secondary metabolites, including alkaloids, polyketides and non-ribosomal peptides, some of which are potent toxins. Most cyanobacterial genera have either been shown to produce non-ribosomal peptides or have them encoded within their genomes. Early work on the genetics of cyanobacterial toxicity led to the discovery of one of the first examples of hybrid peptide-polyketide synthetases. This enzyme complex directed the production of the cyclic heptapeptide, microcystin, while a homologous gene cluster responsible for the synthesis of the pentapeptide nodularin, provided evidence of genetic recombination and possible gene transfer. More recently, hybrid peptide and polyketide synthetic pathways have been implicated in the production of the alkaloid cylindrospermopsin, and this information, in-turn, has provided the first evidence of genes involved in bacterial non-terpene alkaloid biosynthesis. Candidate gene loci involved in saxitoxin production have been identified in diverse cyanobacteria. Genomic information has also indicated the cellular regulators of cyanotoxin production, as well as associated transport mechanisms. Understanding the role of these toxins in the producing microorganisms and the responses of their genes to the environment may suggest the means for controlling toxic bloom events. Exploiting what we refer to as toxins, such as microcystin, is the beginning of the unlimited potential in natural product biosynthetic engineering for the creation of unnatural antibiotics, antivirals, and immunosuppressants. Current directions in drug design and sustainable production, bioprospecting, and ethnopharmacology will be discussed as outcomes of this work.

## **PL13: Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process**

**Roberto De Philippis**

Department of Agricultural Biotechnology, University of Florence, Florence, Italy

Microorganisms can remove metals from the surrounding environment with various mechanisms, either as metabolically mediated processes or as a passive adsorption of metals on the charged macromolecules of the cell envelope. A wide range of microorganisms, both eukaryotes and prokaryotes, has been investigated for the removal of heavy metals from water solutions and promising results have been obtained for a number of them, including cyanobacteria. Owing to the presence of a large number of negative charges on the external cell layers, exopolysaccharide (EPS)-producing cyanobacteria have been considered very promising as chelating agents for the removal of positively charged heavy metal ions from water solutions and an increasing number of studies on their use in metal biosorption have been published in recent years [1].

In this lecture, the attention will be mainly focused on the studies aimed at defining the molecular mechanisms of the metal binding to the polysaccharidic exocellular layers. From the results so far published, it is possible to infer that the mechanisms of interaction between the EPS-producing cyanobacteria and metals are very complex, being involved in this process a large number of factors such as: (i) the chemical and morphological features of microbial cells, (ii) the chemical characteristics of the EPS, (iii) the chemical and physical properties of metals and (iv) their interactions with the other compounds in solution and, finally, (v) the device and (vi) the operational conditions utilized in the treatment.

In the lecture will also be reviewed the few attempts so far available of using EPS-producing cyanobacteria for metal biosorption at pilot scale and with real wastewaters. The most important positive aspects emerging from the studies on the use of EPS-producing cyanobacteria for the removal of metal from water solutions are the very good metal uptake observed in many cases and the possibility to recover the metals from the biomass at the end of the biosorption process. On the other side, the main drawbacks are the costs for producing the biomass and the much slower growth rates of phototrophic microorganisms in comparison with chemo-heterotrophs. However, the current studies on the genes involved in the biosynthesis of EPS in cyanobacteria [2] might give the opportunity to introduce specific alterations on the composition/structure of the polymers, thus producing tailored polysaccharides with much higher specificity and/or metal biosorption capacity.

[1] De Philippis R., Colica G., Micheletti E. (2011) *Appl. Microbiol. Biotechnol.* 92: 697-708; [2] Pereira S., Zille A., Micheletti E., Moradas-Ferreira P., De Philippis R., Tamagnini P. (2009) *FEMS Microbiol. Rev.* 33: 917-941.

## PL14: CyanoGEBa: The Cyanobacterial Volume of the Genomic Encyclopedia of Bacteria and Archaea

Cheryl Kerfeld<sup>1,2,3</sup> and Muriel Gugger<sup>4</sup>

<sup>1</sup> DOE Joint Genome Institute, Lawrence Berkeley National Laboratory Berkeley, CA. USA; <sup>2</sup> Department of Plant and Microbial Biology, University of California, Berkeley, CA. USA; <sup>3</sup> Berkely Synthetic Biology Institute, Berkeley, CA. USA; <sup>4</sup> Collection des Cyanobactéries, Institut Pasteur, Paris, France

In contrast to many other phyla, we have a very limited understanding of the Cyanobacteria at the genomic level. Moreover, the Cyanobacteria have been subjected to biased sequencing choices, skewing our understanding of the genomic basis of cyanobacterial diversity. We have sequenced 54 diverse cyanobacterial genomes, including organisms from Subsections II and V which previously were without sequenced representatives, in an effort to further improve and balance our knowledge of the phylum. The CyanoGEBa organisms were chosen based on 1) their phylogenetic placement in poorly sampled regions of the cyanobacterial tree, 2) their relevance to the cyanobacterial community (i.e. type strains), and 3) the novelty of their physiology. Analyses of all available cyanobacterial genomes after the inclusion of the CyanoGEBa dataset reveals previously unrecognized metabolic potential and insights into the genomics basis of morphological diversity and evolution.

## PL15: Diversity and niche specialization of green sulfur bacteria

Jörg Overmann

Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

Currently, a total of 323 individual 16S rRNA gene sequences of green sulfur bacteria (*Chlorobiaceae*) are available in the databases. These sequences originate from cultivation based and culture independent studies. Rarefaction analysis and statistical projection indicate that the known sequences cover most of the diversity of green sulfur bacterial species in the sampled habitats. Given the very similar physiology that has been determined by standard methods for the described *Chlorobiaceae*, the considerable phylogenetic diversity could be functionally redundant rather than the result of niche specialization. Yet, biogeographic analyses revealed that some of the phylotypes are ubiquitous, providing indirect evidence for a broader environmental tolerance of these *Chlorobiaceae*. Others have so far been observed only in single or few environments and seem to occupy a very narrow niche. A monospecific population of the extremely low-light adapted *Chlorobium* sp. BS-1 has so far been found exclusively at a water depth of 100 m in the Black Sea. The mechanisms of low-light adaptation comprise an increased size of the photosynthetic antenna, a reduced maximum growth rate as well as a significantly decreased maintenance energy requirement. The decrease in maintenance energy requirements is not caused by a reduction of genome size (2.74 MBp). One feature of BS-1 is an unusually high transcript stability that was detected by monitoring intracellular levels of the ribosomal internal transcribed spacer. As another specialization, green sulfur bacteria have adapted to life in a highly structured association with chemotrophic *Betaproteobacteria*. Based on genome comparisons with free-living relatives, only 186 unique ORFs are involved in niche specificity, including putative symbiosis genes that are closely related to bacterial virulence genes such as large haemagglutinin-like proteins, putative haemolysins or RTX toxins. These genes most likely originated from proteobacterial pathogens and were transferred laterally. Transcriptomic and proteomic analyses of consortia and pure epibiont cultures revealed that 352 genes are differentially regulated. Most of the regulated genes encode components of central metabolic pathways and may serve to fine-tune the symbiotic interaction in '*C. aggregatum*'. Since most of these genes also present in free-living relatives, the ancestor of the epibiont might have been preadapted to symbiotic interactions.

[1] Marschall, E., Jogler, M., Henssge, U., Overmann, J. (2010) Large scale distribution and activity patterns of an extremely low-light adapted population of green sulfur bacteria in the Black Sea. *Environ. Microbiol.* 12: 1348-1362; [2] Wenter, R., Hütz, K., Dibbern, D., Reisinger, V., Li, T., Plösch, M., Eichacker, L., Eddie, B., Hanson, T., Bryant, D., Overmann, J. (2010) Expression-based identification of genetic determinants of the bacterial symbiosis in '*Chlorochromatium aggregatum*'. *Environ. Microbiol.* 12: 2259-2276

## PL16: How do we know what we don't know?: Lessons from genomics and metagenomics of uncultivated nitrogen-fixing cyanobacteria

Zehr, J. P.<sup>1</sup>, Thompson, A. W.<sup>1</sup>, Robidart, J.<sup>1</sup>, Foster, R. A.<sup>2</sup>, Hilton, J.<sup>1</sup> and T. A. Villareal<sup>3</sup>

<sup>1</sup> Department of Ocean Sciences, University of California, Santa Cruz, California 95064 USA; <sup>2</sup> MPI for Marine Microbiology, Celsiusstr. 1, D-28359 Bremen, Germany; <sup>3</sup> Marine Science Institute and Dept. of Marine Science, The University of Texas at Austin, 750 Channel View Dr., Port Aransas, Texas 78373 USA

Nitrogen is one of the key nutrients that limit the productivity of the oceans. Biological nitrogen fixation (BNF) is an important source of nitrogen for the surface waters of oligotrophic oceans. Oceanic BNF was believed to be primarily due to the marine cyanobacterium *Trichodesmium* and diatom symbionts, until the discovery of nitrogenase genes from other bacteria and cyanobacteria. Using gene fragments as tracers, cell sorting techniques coupled with genomic and metagenomic approaches have shown that there are surprising new modes of nitrogen metabolism, photosynthesis and symbiosis in diazotrophic planktonic assemblages. Nitrogen-fixing cyanobacteria have diverse physiologies and ecologies, but little is known about how they are differentially distributed in the ocean. Our ability to evaluate ecological distributions is limited by the natural variability of the oceans in space and time, requiring the development of remote instrumentation to link genomics, physiology, and ecology for understanding the ecological controls on nitrogen-fixing cyanobacteria in the world's oceans.



**PL17: The cyanobacteria from arid soil crusts: a pulsed mode of existence of global proportions****Ferran Garcia-Pichel<sup>1,2</sup>**<sup>1</sup> School of Life Sciences, Arizona State University, Tempe, Arizona, USA; <sup>2</sup> Life Sciences Division, Lawrence Berkeley National Lab, Berkeley, California, USA

Where plant cover is restricted and light reaches unimpeded the surface of the soil, one often finds phototrophic microbial communities that are dominated by cyanobacteria. These are known as soil crusts. They become important components of the ecosystem, particularly in arid lands. While relatively inconspicuous to microbiologists, they account for some 50 x10<sup>12</sup> g C of phototrophic biomass globally, second only among phototrophs to the oceanic planktonic forms. While they are only metabolically active during short pulses of water availability, they contribute significantly to the global N and C cycles, and, according to recent findings, they have been doing so since the middle Proterozoic. Several groups of phylogenetically distinct, motile, bundle or rope-forming, filamentous cyanobacteria are the primary colonizers in these crusts, in what is a clear case of convergent evolution to optimize soil-stabilizing capacity. As a result of their impact, soil crusts become the primary force against soil erosion in arid lands. Biogeographic, molecular surveys indicate that a continental-scale replacement of two of the major pioneer cyanobacteria takes place in N America: *Microcoleus vaginatus* dominates in Northern arid lands, whereas *M. steentrupii* does so in Southern deserts. Culture-based experiments indicate that this replacement has to do with differential adaptations to temperature. In fact, similar latitudinal/climatic replacements can be demonstrated for secondary colonizers: the heterocystous *Nostoc*, *Scytonema* and *Tolypothrix*. It is clear that specific adaptations like the synthesis of UV sunscreens, the use of hydrotaxis and phototaxis, bundle-formation, and the secretion of abundant EPS, contribute to cyanobacterial success in these habitats. But several aspects of their biology remain mystifying, particularly those that have to do with gene regulation in the face of unpredictable environmental cues imposed by cycles of wetting and drying. We are using meta-transcriptomics of natural populations to unravel gene expression patterns that might shed some light into this aspect of their biology.

**PL18: Sensing and signaling systems in *Nostoc punctiforme*****Jack Meeks<sup>1</sup>, Nathan Rockwell<sup>2</sup>, Daniela Ferreira<sup>1</sup>, Elsie Campbell<sup>1</sup>, Doug Risser<sup>1</sup> and J. Clark Lagarias<sup>2</sup>**<sup>1</sup> Department of Microbiology, University of California, Davis, CA 95616; <sup>2</sup> Department of Molecular and Cellular Biology, University of California, Davis, CA 95616

*Nostoc punctiforme* is an exceptional experimental system for identification of the subtle complexities of cyanobacteria. *N. punctiforme* has an unparalleled breadth of phenotypic traits, ranging from multiple vegetative cell developmental alternatives in a multicellular organization, to synthesis of secondary metabolites such as nostopeptolides, geosmin, scytonemin and mycosporines, and to nitrogen-fixing symbiotic association with terrestrial plants. Expression of the full extent of these traits is conditional, dependent on responses to environmental and cellular signals. The 9.06 Mbp *N. punctiforme* genome is being analyzed by its sequence and organization, expression through transcriptomics and proteomics, and functional genetic analysis via gene deletion and replacement. Consistent with its extensive, conditionally expressed phenotypes, the *N. punctiforme* genome encodes about 400 sensory transduction proteins; one-third of these genes are differentially transcribed during the differentiation of motile hormogonium filaments. These signal transduction proteins have a highly modular organization, many of which contain multiple CBS, CHASE, GAF, PAS or peri-plasmic sensing domains, one or both a histidine (HK) or serine/threonine (PK) protein kinase domain, and response regulator receiver, Hpt, adenylate/guanylate cyclase, GGDEF and EAL as output domains. We are currently focusing on signal transduction systems that utilize bilins, primarily phycocyanobilin, bound to GAF domains as the sensor. These photo-receptors are classified as cyanobacterial phytochrome HKs (Cph; 6 encoded) or cyanobacteriochromes (CBCR; 16 encoded), 11 of which are HKs, and 2 are methyl accepting chemotaxis proteins (MCP). In addition, the genome encodes 4 HKs and 2 MCPs with related GAF domains that do not bind a chromophore. A brief overview of these proteins will be presented. Two CBCR-MCPs are homologous to PixJ which, in part, modulates phototaxis in *Synechocystis* sp. strain PCC 6803. Mutation analysis indicates the closest *N. punctiforme* PixJ homolog (in terms of sequence and organization) is not its primary phototaxis sensor. Rather, mutation of another homolog, called PtxD, results in motile, but non-phototactic hormogonia of *N. punctiforme*. ptxD is a member of an 8 gene cluster encoding other components of a typical chemotaxis-like signal transduction system, but lacking genes encoding proteins for methylation and demethylation of the MCP. In addition to HAMP and MCP signaling domains, PtxD has 7 GAF domains, 6 of which bind chromophores and possess different light absorption photocycles. The discussion will focus on wavelength-dependent hormogonium phototactic behavior in the context of the light absorption properties of PtxD and related CBCRs.

## PL19: Alternative electron transfer pathways in cyanobacteria thylakoid membrane

**Eva-Mari Aro**

Department of Biochemistry and Food Chemistry, University of Turku, FIN 20014, Turku, Finland

Cyanobacteria thylakoid membrane has unique electron transfer pathways, which are absent from higher plants. One example is the family of flavodiiron proteins (FDP). FDPs are present in all B-cyanobacteria but the size of the family varies depending on the species. FDPs have gradually disappeared during evolution of higher plants. *Synechocystis* sp. PCC 6803 genome contains four genes (*flv1*, *flv2*, *flv3* and *flv4*) encoding FDPs (*Flv1*, *Flv2*, *Flv3* and *Flv4*, respectively) and *Anabaena* 7120 genome has six *flv* genes. FDPs form a complex group of enzymes, which in many strict and facultative anaerobes function in detoxification of O<sub>2</sub> and/or NO. Cyanobacterial FDPs share sequence homology with those in Bacteria and Archaea but additionally have a specific C-terminal extension. *Synechocystis* FDPs are strongly expressed in low Ci condition, i.e. upon limitation of electron flow by terminal electron acceptors as well as under iron starvation, high light and other stress conditions.

*Flv1* and *Flv3* proteins function in the Mehler-like reaction to deliver electrons to molecular oxygen on the reducing side of photosystem I. In WT, up to 20% of electrons derived from water are directed to molecular oxygen under air level CO<sub>2</sub> and this is considerably enhanced upon severe limitation of Ci. Under the latter condition, also the photorespiratory O<sub>2</sub> uptake is enhanced and up to 60% of electrons originated from water are targeted to molecular oxygen. Heterocyst and vegetative cells of *Anabaena* have specific pairs of *Flv1* and *Flv3*-like proteins in both cell types.

The *flv4-flv2* operon encodes three proteins, the *Flv4*, *Sll0218* and *Flv2* proteins, and is negatively regulated by the *AbrB*-like transcription regulator and post-transcriptionally by several antisense RNAs. All three proteins are likely involved in photoprotection of photosystem II under low Ci, high light and other stress conditions. Different from FDPs in anaerobic microbes, the *Flv2* and *Flv4* proteins in cyanobacteria form a heterodimer. Inactivation of the *flv4-flv2* operon distorts the function of PSII and also weakens the transfer of excitation energy from the phycobilisome antennae to PSII.

Beside FDPs, also the multiple NDH-1 complexes mediate cyclic electron flow and perform other alternatives electron transfer reactions in the thylakoid membrane of cyanobacteria. The structural and functional diversity of the NDH-1 complexes in *Synechocystis* 6803 will also be addressed.

## PL20: A Day and a Night in the Life of a Unicellular Diazotrophic Cyanobacterium

**Jana Stöckel, Anindita Bandyopadhyay, Michelle Liberton, Thanura Elvitigala and Himadri B. Pakrasi**

Department of Biology, Washington University, St. Louis, MO 63130, USA

Cyanobacteria are unique among prokaryotes for their ability to perform oxygenic photosynthesis and for their circadian lifestyle. Unicellular cyanobacteria like *Cyanothece* are fascinating organisms that actively use both phases of the diurnal cycle to separate and carry out antagonistic metabolic processes. The intracellular environment oscillates between aerobic and anaerobic states during the day-night period, allowing oxygen-sensitive processes like nitrogen fixation to occur at night while photosynthetic oxygen evolution takes place during the day. This diurnal periodicity gives a highly dynamic profile to these organisms, which can be captured at the genome, transcriptome, proteome and ultrastructural levels. Whole genome analysis of six *Cyanothece* strains revealed that this group of unicellular nitrogen fixing cyanobacteria specialize in metabolic compartmentalization and energy storage, concomitantly accumulating metabolic products in inclusion bodies that are later mobilized as part of a robust diurnal cycle (1, 2). Transcriptomic analyses showed that the existence of these incompatible processes in the same single cell depend on tightly synchronized expression programs involving ~30% of genes in the genome, a phenomenon that also extends to a large extent at the protein level (3). To examine the proteome of *Cyanothece*, we used a high-throughput accurate mass and time (AMT) tag approach and identified a total of 3,616 proteins with high confidence, which is approximately 68% of the predicted proteins based on the sequenced genome (4). We have also examined the dynamic profiles of all of these proteins during a diurnal period (5). Three-dimensional tomographic reconstructions showed that the thylakoid membranes in *Cyanothece* form a dense and complex network that extends throughout the entire cell (6). In particular, the organization of these membranes and various intracellular bodies profoundly change between day and night periods. Together, these studies have provided a comprehensive picture of how a physiologically relevant diurnal light-dark cycle influences the metabolic behavior of a diazotrophic photosynthetic bacterium.

1. Welsh, E. A., et al. (2008) Proc Natl Acad Sci USA 105, 15094-15099; 2. Bandyopadhyay, A., et al. (2011) mBio 2, doi: 10.1128/mBio.00214-11; 3. Stöckel, J., et al. (2008) Proc Natl Acad Sci USA 105, 6156-6161; 4. Stöckel, J., et al. (2011) PLoS One 6, e16680; 5. Aryal, U. K., et al. (2011) BMC Syst. Biol. 5, 105; 6. Liberton, M., et al. (2011) Plant Physiol 155, 1656-1666; [These studies have been supported by United States Department of Energy]

**PL21: Assembling and maintaining the Photosystem II complex in cyanobacteria****Peter J. Nixon**

Sir Ernst Chain Building, Department of Life Sciences, Imperial College London, S. Kensington campus, London, SW7 2AZ, UK

Oxygenic photosynthetic organisms such as cyanobacteria grow because of their ability to use sunlight to extract electrons from water. This vital reaction is catalysed by the Photosystem II (PSII) complex, a large multi-subunit pigment-protein complex embedded in the thylakoid membrane system. Recent results from a number of labs suggest that PSII assembly in cyanobacteria occurs in a step-wise fashion in defined regions of the membrane system, involves conserved auxiliary factors and is closely coupled to chlorophyll biosynthesis [1]. Despite the existence of multiple photoprotective mechanisms *in vivo*, visible light will ultimately cause fatal and irreversible damage to PSII (so-called chronic photoinhibition). Damaged PSII can, however, be repaired through the operation of a 'PSII repair cycle', which involves partial disassembly of the damaged PSII complex and the selective replacement of the damaged subunit (predominantly the D1 subunit) by a newly synthesised copy and reassembly. A key area of current research is to understand how damaged D1 is recognised and removed from the thylakoid membrane. Our recent experimental data support a model in which D1 is degraded in the cyanobacterium *Synechocystis* sp. PCC 6803 by a hetero-oligomeric complex composed of two different types of FtsH subunit (FtsH2 and FtsH3), with degradation proceeding from the N-terminus of D1 in a highly processive reaction [2]. We postulate that a similar mechanism of D1 degradation also operates in chloroplasts. Deg proteases are not required for D1 degradation in *Synechocystis* 6803 but members of this protease family appear to play a supplementary role in D1 degradation in chloroplasts.

[1] Komenda, J., Sobotka, R. and Nixon, P. J. (2012) Assembling and maintaining the Photosystem II complex in chloroplasts and cyanobacteria. *Curr. Opin. Plant Biol.* 15, 245-251; [2] Boehm, M., Yu, J., Krynicka, V., Barker, M., Tichy, M., Komenda, J., Nixon, P. J. and Nield J. Subunit organization of a hetero-oligomeric thylakoid FtsH complex involve Photosystem II repair (submitted)

**PL22: Cyanobacteria and eukaryotes: do they dialogue or not?****Vitor Vasconcelos<sup>1,2</sup>**<sup>1</sup>CIMAR/CIIMAR/ Laboratory of Ecotoxicology, Genomics and Evolution, University of Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal;<sup>2</sup>Departament of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

Secondary metabolites produced by cyanobacteria are many times referred as being part of a communication system that enables them to gain advantages in their environment. This can be more important in the case of symbiotic cyanobacteria in lichens, sponges or other invertebrates, where the close relationship between the prokaryote and the eukaryotic host facilitates this dialogue. Nevertheless, even in open water the release of secondary metabolites, some of them with toxic properties to eukaryotes may also be seen as an example of chemical war that eliminates competitors and/or predators, allowing cyanobacteria to form blooms. The diversity of secondary metabolites and of their chemical pathways does not allow us to define a specific pattern for the behavior of cyanobacteria and their relationship with eukaryotes. Some interesting examples of these metabolites such is the case of the cyanotoxin cylindrospermopsin shows us that sometimes the toxic properties, that we clearly see by the occurrence of human intoxication episodes, may be only one of the aspects of their mechanisms of action. In this presentation we will refer to the diversity of secondary metabolites produced by cyanobacteria, namely the cyanotoxins and allelopathic substances and the potential advantages of these compounds to the producer organisms and/or their hosts. Evidences of their activities towards eukaryotic species will be discussed as also the ecological aspects associated with some of these compounds will be highlighted.

# ORAL COMMUNICATIONS



# ORAL COMMUNICATIONS

## OC1: Evolution of photosynthesis in marine *Roseobacter*s

Michal Koblížek<sup>1</sup>, Aleš Horák<sup>2</sup> and Miroslav Oborník<sup>1,2</sup>

<sup>1</sup>Institute of Microbiology CAS, Opatovický mlýn, 379 81 Třeboň, Czechia Rep.; <sup>2</sup>Institute of Parasitology CAS, Branišovská 31, 370 05 České Budějovice, Czech Rep.

The *Roseobacter* clade constitutes a large part of marine microbial communities. Although it was originally described as containing phototrophic bacteria, many isolates of strictly heterotrophic strains related to the *Roseobacter* clade reveal that this cluster is composed of both photoheterotrophic as well as purely heterotrophic species. Such functional diversity raises the question of the evolutionary origin of the clade. Potentially two evolutionary scenarios can be put forward: (1) the progenitors of *Roseobacter* species were originally photoautotrophic, but they lost part or all of their photosynthetic genes over time, or (2) they were originally heterotrophs adopting part of the photosynthetic genes via the horizontal gene transfer. The tentative origin of *Roseobacter* species was deduced from available genomic data as well as single gene sequences of selected isolates. Our analyses suggest that the photosynthetic genes were not acquired *via* horizontal gene transfer, but rather all the *Roseobacter* species descend from ancient photoautotrophic organisms, which have progressively lost their photosynthetic properties forming photoheterotrophic or strictly heterotrophic species. In addition, we attempted to estimate when *Roseobacter* species diverged from photoautotrophic species belonging to Rhodobacterales using molecular dating. Performed analyses provided similar estimates for both photosynthetic ( $935 \pm 231$  million years ago) and nonphotosynthetic genes ( $901 \pm 171$  million years ago), which suggests that *Roseobacter* species present in today's ocean diverged from photoautotrophic *Rhodobacterales* after the oxygenation of Earth's atmosphere at the end of Proterozoic era.

## OC2: Morphological, molecular and ecological diversity of *Anabaena* spp. (cyanobacteria) without gas vesicles

Eliška Zapomělová<sup>1,2</sup>, Klára Řeháková<sup>1,3</sup>, Andreja Kust<sup>4</sup>, Alena Lukešová<sup>5</sup>, Olga Skácelová<sup>2</sup>

<sup>1</sup>Biology Centre of AS CR, v.v.i., Institute of Hydrobiology, Na Sádkách 7, CZ-37005 České Budějovice, Czech Republic; <sup>2</sup>University of South Bohemia, Faculty of Science, Branišovská 31, CZ-37005 České Budějovice, Czech Republic; <sup>3</sup>Institute of Botany, AS CR, Dukelská 135, CZ-37982 Třeboň, Czech Republic; <sup>4</sup>Cara Hadrijana 8/A, 31000 Osijek, Croatia; <sup>5</sup>Biology Centre of AS CR, v.v.i., Institute of Soil Biology, Na Sádkách 7, CZ-37005 České Budějovice, Czech Republic

We screened for the occurrence of benthic and soil *Anabaena* spp. across a wide spectrum of habitats. We studied discharges of mining water of high conductivities (2000-7000  $\mu$ S), coal mining pits, mineral springs, mofettes, natural saline marshes, common fishponds and pools, reservoirs, peatbogs, sand pits, freshwater streams, limestone caves, and various soil types. Most of the sampling sites were situated in the Czech Republic; however, we also examined several samples from Slovakia, Ireland, France, India, USA and South Africa. Detailed morphological characterization was done and the 16S rRNA gene was sequenced in 62 clonal strains from 39 different localities. This cyanobacterial group was recognized highly polyphyletic and some representatives were mixed with planktonic strains of the genus *Dolichospermum* in phylogenetic trees. We identified even more molecular clusters of non-gas-vacuolate *Anabaena* than have been published so far (Halinen et al. 2008). Taxonomic status of the genus *Anabaena* and its relationships to other cyanobacterial genera, incl. *Wolleea*, were discussed.

Halinen, K., Fewer, D.P., Sihvonen, L.M., Lyra, C., Eronen, E., Sivonen, K. (2008): Genetic diversity in strains of the genus *Anabaena* isolated from planktonic and benthic habitats of the Gulf of Finland (Baltic Sea). *FEMS Microbiology Ecology* 64: 199-208.

## OC3: The diversity of anoxygenic phototrophic bacteria in the Buryat and Mongolian thermal springs

V.M. Gorlenko<sup>1</sup>, A.M. Kalashnikov<sup>1</sup>, M.V. Sukhacheva<sup>2</sup>, B.B. Namsaraev<sup>3</sup>, D.D. Barhutova<sup>3</sup>, F.N. Panteleeva<sup>2</sup>, V.A. Gaysin<sup>2</sup> and B.B. Kuznetsov<sup>2</sup>

<sup>1</sup>Winogradsky Institute of Microbiology, Russian Academy of Sciences; <sup>2</sup>Bioengineering Center, Russian Academy of Sciences; <sup>3</sup>Institute of General and Experimental Biology, Siberian Branch, Russian Academy of Sciences

Thermal springs with a gradual decrease in temperature from extremely high to moderate are a convenient natural model for a study of changes in the structure of phototrophic communities. We have performed a complex study of the species composition of communities of phototrophic microorganisms in microbial mats of six alkaline thermal springs of Baikal rift zone and one in Central Mongolia from different temperature zones (from 84 °C till 20 °C). In the samples of microbial mats of the springs with temperature up to 72 °C were found filamentous anoxygenic phototrophic bacteria (FAPB) according the results of samples studies under the light microscope.

In phototrophic communities nonsulfur purple bacteria (NPB) were present as well as cyanobacteria. Some of them were found in samples of bacterial mat formed at temperatures above 50 °C. The purple nonsulfur bacteria *Rhodospirillum* sp., *Rhodopseudomonas* sp., *Rhodobacter* spp., *Blastochloris* sp. and *Rhodomicrobium vannielii* were isolated due to incubation the samples at 32 °C. The last two species due formation of cysts and exospores has to ability to resistance to survive under high temperatures environment. Purple sulfur bacteria *Chromatium* sp. and *Thiocapsa* sp. were present in bacterial mats only in mats with relatively low-temperature (42 °C - 20 °C). Truly thermophilic purple sulfur and nonsulfur bacteria in the studied samples have not been identified. For identification of FAPB in nature samples were used a system of PCR-primers developed by us for detection of 16S rRNA genes and of genes *pufLM* specific for FAPB. Found that in high temperature areas (72 °C - 45 °C) *Chloroflexus aurantiacus* dominated among FAPB. *Roseoflexus* sp. also was detected in mats of several thermal springs. Mesophilic *Oscillochloris* sp. and unknown mesophilic FAPB were detected in the low-temperature zones of the springs. The monocultures of thermophilic and mesophilic of FAPB were isolated. The new mesophilic FAPB are phylogenetically distinct from others described species of phototrophic bacteria of phylum Chloroflexi. As a result, we discuss possible ways of evolution of a FAPB.

## OC4: Phylogeny and Biogeography of the Cyanobacteria *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*

**Cristiana Moreira<sup>1,2</sup>, Vitor Vasconcelos<sup>1,2</sup> and Agostinho Antunes<sup>1,2</sup>**

<sup>1</sup>CIMAR/CIIMAR/ Laboratory of Ecotoxicology, Genomics and Evolution, Rua dos Bragas, 289, 4050-123 Porto, Portugal; <sup>2</sup>Faculty of Sciences, University of Oporto, Rua do Campo Alegre, 4169-007 Porto, Portugal

Cyanobacteria are prokaryotic photosynthetic microorganisms that exist on Earth for over three billion years. Inhabiting a diverse range of ecological niches cyanobacteria have a high research interest in particular due to their negative impact on the aquatic ecosystems through the production of dense blooms and the release of toxic compounds that can affect both humans and animals. Among these are two of the most studied toxic cyanobacteria species: *Microcystis aeruginosa* and *Cylindrospermopsis raciborskii*. With different life histories *M. aeruginosa* is considered a cosmopolitan species while *C. raciborskii* was first described as a tropical species. However is nowadays found in both subtropical and temperate environments being considered an invasive species. Currently the knowledge on the genetic diversity, population structure and phylogeography in a worldwide scale for these two species is limited and the investigations regarding their origin and evolution are mostly unknown. Biogeography patterns are also essential to understand at the intraspecific level the geographical relatedness of the species within these species and ultimately comprehend what types of phenomena are involved in their distribution (e.g. dispersion, population expansion/contraction, local extinction, etc.). Therefore in this study we have assessed the phylogenetic relationships within these two species by comparing worldwide isolates obtained from all of the five continents using a concatenated system comprehending four distinct genetic markers. Our results provide valuable insight to understand how these species have evolved through time, its population structure and how biogeographic patterns can explain the current distribution for these two cyanobacteria species.

This research was funded by the PTDC/AAC-AMB/104983/2008 (FCOMP-01-0124-FEDER-008610) and PTDC/AAC-CLI/116122/2009 (FCOMP-01-0124-FEDER-014029) projects from Fundação para a Ciência e Tecnologia (FCT), and by the PhD fellowship to Cristiana Moreira (Ref. SFRH/BD/47164/2008) from FCT.

## OC5: Characterization of Marine and Freshwater Photosynthetic Communities Cultured Using LEDs

**Roland Liu<sup>1</sup>, Erica Chin<sup>1</sup>, Clay Gimenez<sup>1</sup>, Jean Huang<sup>1</sup>**

<sup>1</sup>F. W. Olin College of Engineering, Needham, MA, USA

This research examines the diversity of anoxygenic photosynthetic bacteria grown from marine and freshwater environments cultured by wavelength selection using LEDs. The cultures were provided either malate or cellulose as the carbon source, ammonium or dinitrogen gas as a nitrogen source, and cultures containing inoculum from marine or freshwater environments were divided and incubated at light wavelengths ranging from 362nm-1050nm. Specific light wavelengths were associated with cultivation of photosynthetic bacteria with particular bacteriochlorophyll pigments. Across a range of nutrient conditions, 880nm and incandescent light cultured predominantly bacteriochlorophyll a containing photosynthetic bacteria, 720nm and 750nm wavelengths cultured bacteriochlorophyll c and d containing phototrophs, 505nm and 610nm resulted in cultivation of either red or green photosynthetic bacteria, and 1050nm cultured bacteriochlorophyll b containing photosynthetic bacteria. Use of LEDs readily cultured green sulfur bacteria. A community was enriched that grows at 590nm. 16SrDNA inventories showed that the community contains both *Chlorobaculum sp.* and *Prosthecochloris sp.* Spectral analysis of this culture indicated absorbance of the major photopigment at 760nm. A *Pelodictyon sp.* that produces copious amounts of a polymer was cultured at 720nm. This poster will present characterization and comparisons of the biochemical and genetic diversity of marine and freshwater photosynthetic communities that have been cultured.

## OC6: A novel, YrdC-like, carbon-stress regulator, active cyclic electron flow at high light intensity, and isoprene production in *Synechococcus sp. PCC 7002* cyanobacteria

**Toivo Kallas<sup>1</sup>, George L. Weir IV<sup>1</sup>, Justin Zangl<sup>1</sup>, Stephanie Krueger<sup>1</sup>, Brandon Thomas<sup>1</sup>, David Furcy<sup>2</sup>, Anuradha Marathe<sup>1</sup>, Matthew E. Nelson<sup>1</sup>, and Eric L. Singasaas<sup>3</sup>**

Departments of <sup>1</sup>Biology-Microbiology and <sup>2</sup>Computer Science, University of Wisconsin, Oshkosh, WI, USA, and <sup>3</sup>Department of Biology, University of Wisconsin, Stevens Point, WI, USA

Cyanobacteria have evolved efficient mechanisms for carbon acquisition, including CO<sub>2</sub> and HCO<sub>3</sub><sup>-</sup> transporters, but their regulation is not fully understood. Microarray studies of *Synechococcus sp. PCC 7002* revealed a hypothetical *yrdC*-like gene (*\_A1732*) up-regulated under low (ambient) CO<sub>2</sub>. Transcription start site mapping via upstream, untranslated region (UTR) probes and bioinformatic tools (CyanoBIKE, MEME, and TOMTOM) revealed YrdC binding sites upstream of the *rbcR/ccmR* (RubisCO/Carbon Concentration Mechanism Regulator) operon (*ccmR-ndhFIII-ndhDIII-cupA*) and other low-CO<sub>2</sub> induced genes. A YrdC knockout mutant grew well in 3% CO<sub>2</sub> but extremely poorly in low CO<sub>2</sub>, indicating that YrdC\_*\_A1732* is an important activator of gene expression in response to CO<sub>2</sub> stress. In other work, we used a pump-probe kinetics spectrophotometer (BioLogic JTS 10) to re-examine cyclic electron transport (ET) in native *Synechococcus 7002* and an NdhF mutant [1] that lacks NAD(P)H dehydrogenase (NDH-I), which is in the known cyclic pathway of cyanobacteria. Cyclic ET around photosystem I (PSI) and the cytochrome (Cyt) *bf* complex provides 'extra' ATP for efficient CO<sub>2</sub> fixation and is implicated in defenses against photodamage [2]. In chloroplasts, a Cyt *bf* - PSI supercomplex catalyzes cyclic flow [3], but such a complex has not been identified in cyanobacteria. At high light intensity (2000 μmol m<sup>-2</sup> s<sup>-1</sup>), cyclic ET increased dramatically accounting for >30% of linear flow in the NdhF mutant. This cyclic ET cannot occur via the NDH pathway and we propose that it involves formation of a Cyt *bf* - PSI supercomplex that is important for adaptation and growth of *Synechococcus 7002* at extreme, high-light intensities. Finally, We have expressed 'codon-optimized' isoprene synthase and 2 C-methyl-D-erythritol 4-phosphate pathway genes in 7002 and obtained production of isoprene (a precursor for synthetic rubber and liquid biofuel) at rates promising for development. The isoprene work is covered by U.S. patent 20110039323 [4] and further IP protection for the cyanobacterial work is being pursued with the UW System, WiSys Technology Foundation. Aspects of these studies will be presented.

[1] Schluchter, W. M., Zhao, J., Bryant, D. A. 1993 J. Bacteriol. 175, 3343-52; [2] Kallas, T. 2012. in Photosynthesis: Plastid Biology, Energy Conversion and Carbon Assimilation. (Eaton-Rye, J.J., Tripathy, B.C., and T.D. Sharkey, Eds), Vol. 34, pp 501-560. Springer, Dordrecht; [3] Iwai M., Takizawa K., Tokutsu R., Okamuro A., Takahashi Y. and Minagawa J. 2010 Nature 464, 1210-1213; [4] Wiberley, A.E., Singasaas, E.L., Sharkey, T.D., U.S. patent 20110039323.



## OC7: Identification and Characterization of Unknown Genes in *Rhodobacter sphaeroides*

Miralem Bekric<sup>1</sup>, Angela Whitaker<sup>1</sup>, Cameron Unverferth<sup>1</sup>, Young-Won Christine Kim<sup>1</sup>, and Aaron Setterdahl<sup>1</sup>

[1] Indiana University Southeast, New Albany, Indiana, USA

Genome sequencing of thousands of organisms has resulted in databases of gene sequences that code for many previously unknown proteins. Sequence analysis of the photosynthetic bacterium *Rhodobacter sphaeroides* genome reveals several uncharacterized proteins that are highly conserved among many bacteria and have yet to be characterized. Genes that have probable, hypothetical, or no known function have been targeted for this study to be systematically validated for function. One such gene, RSP\_2415, has more than 90% sequence identity to a gene of unknown function also found in hundreds of organisms. BLAST analysis reveals two domains of unknown function DUF-404 and DUF-407. DUF-404 is predicted to be structurally similar to a putative glutathionylspermidine synthase. The gene encoding RSP\_2415 has been cloned and expressed to validate the glutathionylspermidine activity. This would be the first time glutathionylspermidine synthase activity is characterized in a photosynthetic prokaryote.

## OC8: Ferredoxin-Dependent Cyanobacterial Enzymes: Mutagenic and Biophysical Studies

Masakazu Hirasawa<sup>1</sup>, Jatindra N. Tripathy<sup>2</sup>, Anurag P. Srivastava<sup>1</sup> and David B. Knaff<sup>1,2</sup>

<sup>1</sup>Dept. of Chemistry & Biochemistry, Texas Tech University, Lubbock, Texas, U.S.A.; <sup>2</sup>Center for Biotechnology & Genomics, Texas Tech University, Lubbock, Texas, U.S.A.

The three-dimensional structure of the ferredoxin-dependent glutamate synthase (hereafter abbreviated as GOGAT) from the cyanobacterium *Synechocystis* sp. PCC 6803 suggests that a loop, located near the domain that contains the enzyme's [3Fe-4S] cluster and FMN prosthetic groups, is likely to be involved in the productive binding of ferredoxin (hereafter abbreviated as Fd), the electron-donating substrate for GOGAT [1]. Regions that correspond to the 27 amino acid loop seen in *Synechocystis* 6803 sp. PCC GOGAT, are present at the same position in all Fd-dependent GOGATs, but are absent in the catalytic subunits of NADPH-dependent GOGATs. Mutagenesis has been used to delete portions of this loop and characterization of the resulting variants has been used to define the Fd-binding site. The binding site for Fd on GOGAT will be compared to that previously determined, using NMR spectroscopy, for the Fd-binding site on the *Synechocystis* sp. PCC 6803 ferredoxin:thioredoxin reductase (FTR) [2-4]. Isothermal titration calorimetry (ITC) has been used to determine the stoichiometries, affinities and thermodynamic parameters for Fd binding by cyanobacterial enzymes, and also for the binding of their electron-accepting substrates. In the case of the Fd-dependent nitrate reductase from the cyanobacterium *Synechococcus* sp. PCC 7942,  $K_d$  was shown to be 80  $\mu$ M for the 1:1 Fd/enzyme complex. Complex formation is stabilized entirely by the highly favorable entropy for the binding process, as the enthalpy of binding is actually unfavorable. The *Synechococcus* sp. PCC 7942 nitrate reductase has also been used to examine the roles of four conserved basic residues (Lys58, Arg70, Lys130, and Arg146). *In silico* structural modeling, site-directed mutagenesis, substrate binding, enzyme kinetics, and prosthetic group analysis have provided insights into the role of these residues in substrate binding and in electron transport.

This work was funded by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through Grant DE-FG02-99ER20346 (to DBK).

[1] van den Heuvel, R.H.H., Ferrari, D., Bossi, R.T., Ravasio, S., Curti, B., Vanoni, M.A., Florencio, F. J., and Mattevi, A. (2002) *J. Biol. Chem.* 277, 24579-24583; [2] Xu, X., Kim, S.-K., Schürmann, Hirasawa, M., Tripathy, J.N., Smith, J., Knaff, D.B., and Ubbink, M. (2006) *FEBS Lett.* 580, 6714-6720; [3] Xu, X., Schürmann, P., Chung, J.-S., Hass, M.A.S., Kim, S.-K., Hirasawa, M., Tripathy, J.N., Knaff, D.B., and M. Ubbink, M. (2009) *J. Am. Chem. Soc.* 131, 17576-17582; [4] Xu, X., Scanu, S., Chung, J.-S., Hirasawa, M., Knaff, D.B., and Ubbink, M. (2010) *Biochemistry* 49, 7790-7797.

## OC9: Spatial distribution of thylakoid membrane complexes and partitioning of reducing power in cyanobacteria

Conrad W. Mullineaux, Samantha J. Bryan and Lu-Ning Liu

School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK

The thylakoid membranes of cyanobacteria house not only the conventional photosynthetic electron transport chain but also a diverse collection of other electron transport complexes interacting with a range of different electron donors and acceptors. This allows multiple possible electron transport pathways. Electron transport routes are crucial for cell physiology. What determines which electron transport pathways predominate, and how is electron transport regulated? Our approach to the problem is based on the use of fluorescent protein (FP) tagging and confocal microscopy to visualise the distribution and dynamics of electron transport complexes in live cells of the model cyanobacteria *Synechocystis* PCC6803 and *Synechococcus* PCC7942. In all cases, the FP-tagged proteins were expressed from the native chromosomal locus to ensure that expression was in context and at physiological levels. Since electron transport is quantifiable, it is easy to check whether function is perturbed by the FP tag. The resolution of fluorescence microscopy is insufficient to give detail at the molecular level, but it does allow very accurate quantification of the total distribution of the protein in the cell, localisation to the cytoplasm, thylakoid membrane or plasma membrane and some sub-localisation in all these cell compartments. In addition we can visualise dynamics, including redistribution of proteins in response to environmental cues. We have successfully FP-tagged the bidirectional hydrogenase (via the HoxF subunit) [1] and the respiratory electron donor complexes NDH-1 (via NdhM) and SDH (via SdhA) [2]. In all these cases, the FP-tagged protein was integrated into the appropriate complex and fully functional. Our imaging studies show that cyanobacterial thylakoid membranes are strikingly heterogeneous on 100-300 nm scales, with distinct islands of functional activity. Furthermore, all three complexes undergo large-scale redistribution in response to light and redox cues. This redistribution correlates with changes in electron transport activity, indicating that distribution of electron transport complexes on the sub-micron scale is a key controller of pathways of electron flow.

[1] Bryan SJ, Burroughs NJ, Boehm M, Eckert C, Yu J, Mullineaux CW, Nixon PJ (2012) Spatial localisation of the hydrogenase in the cyanobacterium *Synechocystis* PCC6803. *in preparation*; [2] Liu L, Bryan SJ, Huang F, Yu J, Nixon PJ, Rich PR and Mullineaux CW (2012) Control of electron transport routes through redox-regulated redistribution of respiratory complexes. *Proc Nat Acad Sci USA under revision*



## OC10: Development of *Leptolyngbya* sp. BL0902 as a new bioengineering platform and improved genetic tools for cyanobacteria

**Arnaud Taton, Ewa Lis, Dawn M. Adin, Guogang Dong, Scott Cookson, Tyler Swinney, Edward King, Steve A. Kay, Susan S. Golden, and James W. Golden**

Division of Biological Sciences, University of California San Diego, La Jolla, California, United States of America

There is great interest in using photosynthetic microorganisms to convert solar energy and CO<sub>2</sub> into fuels and other bioproducts. Cyanobacteria provide an excellent platform for these purposes [1] but experiments have often been carried out with easily manipulable laboratory models rather than with organisms of a greater potential for practical use. A search for strains with superior growth traits led to the discovery of *Leptolyngbya* sp. BL0902, which shows robust growth in a range of temperatures, salt and urea concentrations, alkalinity, and at high solar irradiance. Its growth-rate can rival that of *Arthrospira* strains, and importantly it showed culture stability in large outdoor ponds. *Leptolyngbya* accumulates higher lipid content and a higher proportion of mono-unsaturated fatty acids than *Arthrospira* strains. The suitability of *Leptolyngbya* sp. BL0902 as a platform for biotechnological purposes was established by demonstrating efficient conjugal transfer from *E. coli* of a broad host range self-replicating plasmid, the expression of an antibiotic resistance marker and a reporter gene, and transposon tagging [2]. We are currently developing genetic tools for *Leptolyngbya* sp. BL0902 and other cyanobacterial strains based on the idea that improved, modular, standardized and well-characterized genetic tools will be required to exploit diverse production strains [3]. Inspired by the recent development of synthetic biology technologies, we devised a strategy that allows the efficient construction of modular vectors from up to 4 donor vectors, each vector harboring an essential module. The resulting modular vectors are designed for the parts to be easily replaced or additional parts to be easily inserted. Different types of vectors can also be assembled, including autonomously replicating vectors and suicide vectors for gene knockout and gene expression from the chromosome. A library of donor vectors carrying standardized parts is currently being made. It includes origins of replication for *E. coli*, origins of transfer for conjugation, origin of replication and neutral sites for various cyanobacteria, antibiotic markers, expression cassettes with different promoters and tags, and reporter cassettes. Assembled modular vectors will allow a thorough characterization of different parts in a number of cyanobacterial strains.

[1] Ducat et al 2011, Trends Biotechnol 29: 95–103; [2] Taton et al 2012, PLoS ONE 7: e30901; [3] Huang et al. 2001, Nucleic Acids Res. 38, 2577–2593.

## OC11: Hypothetical proteins to unknown cellular mechanisms: with emphasis on a novel transcription factor of heat shock genes in *Synechocystis* sp. PCC6803

**Pilla Sanakara Krishna<sup>1</sup>, M. Karthik Mohan<sup>1</sup>, B. Radharani<sup>1</sup>, Iwane Suzuki<sup>3</sup>, J.S.S.Prakash<sup>1,2</sup>**

<sup>1</sup>Department of Plant Sciences, School of Life Sciences, University of Hyderabad, Hyderabad, India; <sup>2</sup>Department of Biotechnology, School of Life Sciences, University of Hyderabad, Hyderabad, India; <sup>3</sup>Faculty of Life and Environmental Science, University of Tsukuba, Tsukuba, Japan

Genome of *Synechocystis* sp. PCC6803 has 50% of its genes (1620) encode hypothetical proteins. Out of these, we have identified 34 functionally important hypothetical proteins, based on their conservation, stress regulation and conservation in their transcriptional regulation. Out of these conserved stress responsive genes, a gene *sll1130*, which codes for an hypothetical protein, was strongly repressed upon upshift in temperature and conserved in both gram negative and positive bacteria. With this clue, we proceeded to elucidate the functional role of Sll1130 during heat stress. During heat acclimation, majority of heat shock proteins are known to play important functional roles, especially in maintaining quality control of proteins, refolding of heat denatured proteins, stabilization of the protein structure and solubilization of aggregated proteins. However regulation of such important heat shock genes is poorly understood in bacteria. So far, well known heat responsive transcriptional regulator that negatively regulates the expression of certain heat shock genes is HrcA/CIRCE system. In this presentation, we show Sll1130, a functionally uncharacterized protein, as novel heat responsive transcription factor, that regulates the expression of several important heat shock genes in *Synechocystis* sp. PCC6803. Disruption of this hypothetical protein exhibited an increased tolerance to heat stress. Heat shocked mutant cells recovered much faster than wild type cells. 30% of total wild type cells were viable after 50°C heat shock for 30 min and then subsequent recovery at 34°C for 48 h. In contrast, 70% of total mutant cells remained viable. DNA microarray analysis revealed upregulation of major heat responsive genes such as *htpG*, *hspA*, *isiA*, *isiB* and several genes coding for hypothetical proteins due to mutation in *sll1130* gene. qRT PCR analysis is consistent with the Microarray gene expression changes. Over expression and purification, PAGE analysis of Sll1130 indicated that the protein exists as a homo-dimer *in vivo*. We demonstrated that Sll1130 binds to a conserved inverted repeat, which was identified in the upstreams of genes, which were upregulated due to mutation. Both transcript and protein levels of the gene were quickly down regulated upon shift of wild-type *Synechocystis* cells from 34°C to 42°C, suggesting Sll1130 as a novel heat responsive transcriptional regulator, negatively regulating the expression of heat shock genes in *Synechocystis*.

## OC12: Ecology of Coastal Cyanobacterial Mats

**Lucas J. Stal, Henk Bolhuis, Ina Severin**

Department of Marine Microbiology, Royal Netherlands Institute of Sea Research (NIOZ), P.O.Box 140, 4400 AC Yerseke, The Netherlands

Shallow intertidal sandy sediments are often characterized by the development of microbial mats with oxygenic and anoxygenic phototrophic bacteria as the major mat building component in addition to a complex microbial community. This microbial community is the driver of the biogeochemical cycles which are closed in this small-scale microbial ecosystem. We have investigated cyanobacterial mats developing along a tidal gradient and identified three different types of mats that comprised different communities. Here we report on the capacity of these mats to fix dinitrogen. The mats showed distinctly different daily patterns of dinitrogen fixation that was most likely attributed to different types of phototrophic bacteria. We found that salinity was a major determinant of the spatial diversification of the microbial mats in the intertidal gradient. We review the effect of salinity on diazotrophic phototrophs.

### OC13: Microcystin-binding to proteins is part of the oxidative stress response in *Microcystis aeruginosa*

Sven Meissner<sup>1</sup>, Yvonne Zilliges<sup>2</sup>, Jan-Christoph Kehr<sup>1</sup>, Stefan Mikkat<sup>3</sup>, Martin Hagemann<sup>4</sup>, Aaron Kaplan<sup>5</sup>, Thomas Börner<sup>1</sup>, Elke Dittmann<sup>2</sup>

<sup>1</sup>University of Potsdam, Institute of Biochemistry and Biology, Department of Microbiology Potsdam-Golm, Germany; <sup>2</sup>Humboldt University, Institute of Biology, Berlin, Germany; <sup>3</sup>University of Rostock, Medical Faculty, Core Facility Proteome Analysis, Rostock, Germany; <sup>4</sup>University of Rostock, Institute of Biology, Department of Plant Physiology, Rostock, Germany; <sup>5</sup>Department of Plant and Environmental Sciences, Admond Safra Campus, The Hebrew University of Jerusalem, Jerusalem, Israel; edtt@uni-potsdam.de

Microcystin is a cyanobacterial toxin that causes a serious threat to drinking water and recreational lakes worldwide. The peptide is predominantly produced by freshwater cyanobacteria showing mass developments ("blooms") in the summer month. We have used a systematic approach to illuminate a possible physiological function of microcystin. We have performed a global proteomic comparison with extracts of the microcystin producing wild type strain PCC7806 and the  $\Delta mcyB$  mutant. The microcystin deficient mutant  $\Delta mcyB$  showed significant changes in the accumulation of proteins, including several enzymes of the Calvin cycle, phyco-biliproteins and two NADPH-dependent reductases. Coincidentally, we have discovered that microcystin binds to a number of these proteins *in vivo* and *in vitro*. We have found clear evidence for a covalent interaction of the toxin with cysteines of proteins. The microcystin binding to proteins is strongly enhanced under conditions triggering oxidative stress. Correspondingly, the  $\Delta mcyB$  mutant defective in microcystin production exhibited an increased sensitivity towards oxidative stress conditions [1]. We have developed quantification protocols for total microcystin including the protein-bound fraction and the fraction binding to low molecular weight thiols. A careful re-analysis of laboratory strains and field samples revealed a strong dependency of microcystin production on light conditions (that is not seen using standard protocols) and considerable strain specific differences in the amount of microcystin-binding to proteins. Up to 60% of the total microcystin fractions are neglected with current protocols on microcystin quantification and effects of environmental stimuli on microcystin production have to be re-visited. Taken together, our data suggest a redox-dependent, protein-modulating role for microcystin within the producing cell, which represents a new addition to the catalogue of functions that have been discussed for microbial secondary metabolites.

[1] Zilliges, Y., et al., The Cyanobacterial Hepatotoxin Microcystin Binds to Proteins and Increases the Fitness of *Microcystis* under Oxidative Stress Conditions. Plos One, 2011. 6(3).

### OC14: The TCA cycle in cyanobacteria

Shuyi Zhang<sup>1</sup> and Donald A. Bryant<sup>1,2\*</sup>

<sup>1</sup>Dept. of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA; <sup>2</sup>Dept. of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717 USA

The TCA cycle, one of the key pathways of central metabolism, has two main functions in bacteria: it oxidizes two-carbon units derived from acetyl-CoA producing NADH to drive oxidative phosphorylation at night, and it provides essential precursor metabolites (e.g., oxaloacetate, 2-oxoglutarate) that are required for biosynthesis of cellular components. Since 1967, cyanobacteria have been believed to have an incomplete TCA cycle because they lack 2-oxoglutarate dehydrogenase (2-OGDH), which converts 2-oxoglutarate to succinyl-CoA (1, 2). However, mutants of *Synechocystis* sp. PCC 6803 lacking succinate dehydrogenase could still synthesize succinate when 2-oxoglutarate was supplied to the media, which suggested that there must be an alternative way(s) to convert 2-oxoglutarate to succinate (3).

By using genomic analysis, proteomic profiling and experimental verification, we recently identified genes encoding a novel 2-oxoglutarate decarboxylase (2-OGDC) and succinic semialdehyde dehydrogenase (SSADH) in the cyanobacterium *Synechococcus* sp. PCC 7002 (4). Together, these two enzymes convert 2-oxoglutarate to succinate, and thus they functionally replace 2-oxoglutarate dehydrogenase and succinyl-CoA synthetase. More specifically, 2-oxoglutarate is first converted to succinic semialdehyde (SSA) by 2-OGDC (SynPCC7002\_A2770), and SSA is then converted to succinate by SSADH (SynPCC7002\_A2771). These two reactions thus complete the TCA cycle. All cyanobacterial genomes, except those of *Prochlorococcus* and marine *Synechococcus* species, have these two genes (4). Further enzymatic characterization has demonstrated that SSADH is inhibited by high concentrations of its substrate SSA (above substrate:enzyme ratios of 40:1). Mass spectrometry and native gel electrophoresis suggested that high concentrations of SSA modifies some residues of SSADH and consequently decreased the activity of SSADH.

Identification of these two enzymes not only enriches our knowledge of the central metabolic pathways, but also provides new opportunities to produce important industrial materials in cyanobacteria. For example, 1,4-butanediol, an important commodity chemical used to manufacture over 2.5 million tons of valuable polymers annually, could be produced biologically from SSA by several enzymes (5). Specifically, SSA can first be converted to 4-hydroxybutyrate (4-HB) by 4-hydroxybutyrate dehydrogenase; 4-hydroxybutyrate can then be converted to 4-hydroxybutyryl CoA by 4-hydroxybutyryl-CoA transferase; and 4-hydroxybutyryl-CoA can finally be converted to 4-hydroxybutyraldehyde by 4-hydroxybutyryl-CoA reductase. 4-hydroxybutyraldehyde can then be converted to 1,4-butanediol by the endogenous alcohol dehydrogenase in *Synechococcus* sp. PCC 7002. As an initial step, 4-hydroxybutyrate dehydrogenase of *Ralstonia eutropha* H16 was successfully expressed in *Synechococcus* sp. PCC 7002. Preliminary HPLC analyses showed that about 0.7 mM 4-HB was secreted to medium by this strain after 24-h of growth. We are now attempting to express the remaining two genes required to produce 1,4-butanediol in this strain. With further metabolic engineering, we might be able to achieve the ultimate goal of sunlight-driven biofuel and biomaterial production and potentially contribute to producing these materials in a renewable manner.

[1] Smith, A. J., London, J., and Stanier, R. Y. (1967) J Bacteriol 94, 972-983; [2] Pearce, J., Leach, C. K., and Carr, N. G. (1969) J Gen Microbiol 55, 371-378; [3] Cooley, J. W., Howitt, C. A., and Vermaas, W. F. J. (2000) J Bacteriol 182, 714-722; [4] Zhang, S., and Bryant, D. A. (2011) Science 334, 1551-1553; [5] Yim, H., Haselbeck, R., Niu, W., Pujol-Baxley, C., Burgard, A., Boldt, J., Khandurina, J., Trawick, J. D., Osterhout, R. E., Stephen, R., Estadilla, J., Teisan, S., Schreyer, H. B., Andrae, S., Yang, T. H., Lee, S. Y., Burk, M. J., and Van Dien, S. (2011) Nat Chem Biol 7, 445-452

## OC15: The languages spoken in the water body: what determines who is there or the biological role of cyanobacterial toxins

**Aaron Kaplan<sup>1</sup>, Yehonathan Bar Yosef<sup>1</sup>, Omer Murik<sup>1</sup>, Moshe Harel<sup>1</sup>, Ora Hadas<sup>2</sup> and Assaf Sukenik<sup>2</sup>**

<sup>1</sup>Dept. of Plant and Environmental Sciences, Edmond J. Safra Campus, Givat Ram, Hebrew University of Jerusalem, Jerusalem 91904, Israel; <sup>2</sup>Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research, P.O. Box 447, Migdal 14950, Israel

“What determines who is there?” and the reasons for large multiannual fluctuations in phytoplankton biomass and compositions in the water bodies are some of the long standing but nevertheless intriguing biological/environmental questions. Numerous field and laboratory studies and modeling of the data thereof were performed in an attempt to reveal the effects of abiotic and biotic parameters on the temporal and spatial phytoplankton population dynamics. However, failure to understand and predict the intensification of toxic cyanobacterial blooms over the last decade, a matter of growing concern, is just one example of our poor understanding of the complex interactions of these parameters in the real world. The biological role of most of the toxins produced is not known but recent studies shed some light on the roles of microcystins and cylindrospermopsin in inter- and intra-species communication and in nutrient acquisition [1,2]. Laboratory experiments showed that secretion of cylindrospermopsin to the water body by *Aphanizomenon ovalisporum* (hereafter *Aphanizomenon*) induces the PHO regulon in other phytoplankton species, including secretion of alkaline phosphatase, and thereby enhances Pi availability and acquisition by *Aphanizomenon* [1]. Here we examined the reasons for the large multiannual fluctuations in *Aphanizomenon* biomass in the summer-autumn blooms in Lake Kinneret (Sea of Galilee), Israel, focusing on 2009 and 2010. During the summer the total dissolved phosphate concentration in the lake is very low, close to the detection level, limiting the development of phytoplankton. We show that *Aphanizomenon* blooms are associated with a large rise in alkaline phosphatase (APase) activity in the water body. Transcripts of PHO and AOA (involved in cylindrospermopsin biosynthesis) genes in *Aphanizomenon* in Lake Kinneret appeared much earlier in 2010 than in 2009 suggesting that the phytoplankton became phosphate-limited already at the beginning of its summer bloom in 2010 but much later in 2009 [3]. Water inflow and lake water temperatures were significantly higher in 2010 but the incoming nutrients were consumed by the much larger phytoplankton biomass early in 2010 before the beginning of the *Aphanizomenon* bloom. Our analysis of abiotic and biological parameters provides an explanation for the very different development of *Aphanizomenon* populations during 2009 and 2010 and the role of toxin formation in the environment in the inter species competition for the limiting Pi resource.

[1] Kaplan, A., Harel, M., Kaplan-Levy, R.N., Hadas, O., Sukenik, A. E. Dittmann (2012) *Frontier in aquatic microbiology* (in press); [2] Bar-Yosef, Y., Sukenik, A., Hadas, O., Viner-Mozzini, Y. and A. Kaplan (2010) *Curr. Biol.* 20: 1557-1561; [3] Bar-Yosef, Y., Murik O. Sukenik, A. Hadas, O. and A. Kaplan (2012) *Environ Microbiol Rep* (in press)

## OC16: A novel plasmid-borne arsenite and antimonite responsive operon in *Synechocystis* sp. PCC 6803

**Csaba István Nagy, Péter B. Kós, István Z. Vass, Imre Vass**

Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, H-6701 Szeged, P.O. Box 521, Hungary

Arsenic containing minerals are widely spread in the environment and because of its geological and anthropogenic immobilization groundwater contaminations occur across the world, affecting health of millions of people [1]. For most organisms arsenic is harmful, exerting its toxic effect at multiple sites [2]. To cope with its toxicity, most organisms evolved tolerance and resistance systems against arsenic compounds [3]. Arsenic resistance mechanisms encoded by *ars* genes are widely distributed in prokaryotes. In several bacteria more than one detoxification systems exist, encoded in independent operons in order to sequester, transport or decrease the toxic effects of this metalloid.

Besides the chromosomal arsenic resistance operon, *arsBHC* [4], we present another, arsenite- and antimonite-responsive gene cluster termed *arrRSC*T in the cyanobacterium *Synechocystis* sp. strain PCC 6803. This operon is located on the pSYSM plasmid and has an unusual structure and expression pattern. Similarly to most common *ars* operons, the *arrRSC*T is induced in the presence of the trivalent ions As(III) and Sb(III). The deduced protein sequence of *arrR* shows high homology with DNA-binding proteins of the ArsR family. According to this, we constructed an *arrR*-deficient mutant and observed high constitutive expression of *arrS/C/T* genes in this strain. Furthermore, the As(III) concentration dependent DNA binding affinity of ArrR to the putative promoter region was proven via electrophoretic mobility shift assay. We concluded therefore, that the product of the *arrR* gene is the repressor of the *arrRSC*T operon. The ArrT shows high homology with membrane-bound heavy metal transporter proteins. The  $\Delta$ *arrT* deletion mutant showed increased sensitivity to As(III) compared to the wild type strain, which, together with its inducibility by As(III) proves, that the operon is playing role in arsenic resistance.

The *arrRSC*T operon makes part of a transposable genetic element containing an ORF for IS4-like transposase and it is delimited by imperfect inverted repeats. The remnants of this mobile genetic element in the genome of *Synechocystis* sp. strain PCC 6803, suggests an earlier horizontal gene transfer event.

[1] Silver, S., Phung, L. T. 2005. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. Environ. Microbiol.* 71: 599-608; [2] Mukhopadhyay, R., B. P. Rosen, L. T. Phung, and S. Silver. 2002. Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiol. Rev.* 26: 311-325

## OC17: Fluorescence kinetics in a diazotrophic cyanobacterium: evidence for diurnal state transitions and consistent inhibition during nitrogen fixation

Sophie Rabouille<sup>1,2</sup>, Pascal Claquin<sup>3</sup>, Tae Hyun Park<sup>1,2,4</sup>, and Anthony Dron<sup>1,2</sup>

<sup>1</sup>UPMC Univ Paris 06, UMR 7093, LOV, Observatoire océanologique, F-06234, Villefranche/mer, France; <sup>2</sup>CNRS, UMR 7093, LOV, Observatoire océanologique, F-06234, Villefranche/mer, France; <sup>3</sup>Université Caen Basse-Normandie, FRE BIOMEA, F-14032 Caen, France; <sup>4</sup>BIOCORE-INRIA, BP93, 06902 Sophia Antipolis Cedex, France

*Crocospaera watsonii* WH8501 belongs to group B, unicellular diazotrophic cyanobacteria (UCYN) whose important role as nitrogen fixers is now acknowledged [1-4]. In UCYN, where the night-time process of diazotrophy is highly sensitive to oxygen, nitrogen fixation is tightly regulated, both at the transcriptional level and the physiological level, in response to environmental constraints [5-7]. It is known that, as a result, nitrogenase activity is restrained to the dark period and thus timely decoupled from photosynthesis. We continuously monitored fluorescence kinetics in continuous cultures of *C. watsonii* brought to the equilibrium under four different light:dark regimes. Results are discussed in relation to the timing of nitrogenase activity. Photosystem II (PSII) fluorescence reveals a maximum electron transport rate related to the daily irradiance regime, as well as fast regulations through state transitions during the light period. Unreported so far was the night time shutdown of PSII activity. Contrary to what can usually be observed in phototrophs, fluorescence kinetics in *C. watsonii* reveals a decrease in electron transport towards the end of the light period and an absence of fluorescence variation during the dark period, without recovery upon application of light. It is suggested that either phycobilisomes are consistently disconnected from PSII or PSII is deactivated. Results hint toward a tight constraint of oxygen evolution in *C. watsonii* as additional protection of nitrogenase activity.

[1] Zehr JP, Waterbury JB, Turner PJ, Montoya JP, Omoregie E, Steward GF et al. (2001). Unicellular cyanobacteria fix N<sub>2</sub> in the subtropical North Pacific Ocean. *Nature* 412: 635-638; [2] Falcon LI, Cipriano F, Chistoserdov AY, Carpenter EJ. (2002). Diversity of diazotrophic unicellular cyanobacteria in the tropical North Atlantic Ocean. *Appl Environ Microbiol* 68: 5760-5764; [3] Montoya JP, Holl CM, Zehr JP, Hansen A, Villareal TA, Capone DG. (2004). High rates of N<sub>2</sub> fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* 430: 1027-1032; [4] Zehr, J. P. 2011. Nitrogen fixation by marine cyanobacteria. *Trends Microbiol.* 19: 162-173; [5] Webb, E.A., Ehrenreich, I.M., Brown, S.L., Valois, F.W., and Waterbury, J.B. (2009) Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocospaera watsonii*, isolated from the open ocean. *Environ Microbiol* 11: 338-348; [6] Pennebaker, K., K. R. M. Mackey, R. M. Smith, S. B. Williams, and J. P. Zehr. 2010. Diel cycling of DNA staining and *nifH* gene regulation in the unicellular cyanobacterium *Crocospaera watsonii* strain WH 8501 (Cyanophyta). *Environ. Microbiol.* 12: 1001-1010; [7] Shi, T., Ilikchyan, I., Rabouille, S., and Zehr, J.P. (2010) Genome-wide analysis of diel gene expression in the unicellular N<sub>2</sub>-fixing cyanobacterium *Crocospaera watsonii* WH 8501. *ISME J* 4: 621-632.

## OC18: Production of isoprenoids in *Synechocystis* PCC 6803

Elias Englund, Pia Lindberg

Photochemistry and Molecular Science, Department of Chemistry-Ångström, Uppsala University, Uppsala, Sweden

In the project presented here, our aim is to use cyanobacteria to produce hydrocarbons to serve as an alternative to petroleum products. The goal of the project is to over-produce hydrocarbons originating from the isoprenoid biosynthesis, using the unicellular cyanobacterium *Synechocystis* PCC 6803 as a model organism. Isoprenoids, or terpenoids, is a large family of compounds, including carotenoids, tocopherol, phytol, sterols and hormones. There are two biosynthetic pathways leading to the formation of isoprenoids; the mevalonic acid pathway, which operates in the cytosol of eukaryotes and archaea; and the methyl-erythritol-4-phosphate (MEP) pathway, which is of prokaryotic origin and also present in plant plastids [1].

The MEP pathway, as described for *Escherichia coli* [2], converts pyruvate and glyceraldehyde-3-phosphate in a series of steps to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Only a few studies have investigated this pathway in cyanobacteria. While the sequenced genome of *Synechocystis* [3] contains all the genes needed to encode the enzymes involved in the MEP pathway in *E. coli*, some studies have indicated differences in the way the pathway functions [4-6].

IPP and DMAPP are the building blocks for the subsequent formation of all isoprenoids. In a previous study, *Synechocystis* was modified to produce the volatile five-carbon compound isoprene from DMAPP, a reaction catalysed by the enzyme isoprene synthase [7]. In the present project, we are interested in the possibilities for producing other potentially useful isoprenoids, as well as in further investigating the isoprenoid biosynthesis pathway in cyanobacteria. *Synechocystis* is a suitable model organism for such studies, due to the ease with which it can be genetically modified. Results illustrating an example of production of isoprenoids in *Synechocystis* will be presented.

[1] Lichtenthaler, 2000, *Biochem Soc Trans* 28, 785; [2] Rohmer et al., 1996, *J Am Chem Soc* 118, 2564; [3] Kaneko and Tabata, 1997, *Plant Cell Physiol* 38, 1171. [4] Ershov et al., 2002, *J Bacteriol* 184, 5045; [5] Poliquin et al., 2004, *J Bacteriol* 186, 4685; [6] Okada and Hase, 2005, *J Biol Chem* 280, 20672; [7] Lindberg et al., 2010, *Metab Eng* 12, 70



## OC19: Fast and specific termination of nuisance cyanobacteria with hydrogen peroxide

**Hans C.P. Matthijs and Petra M. Visser**

Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

In many natural habitats abundant presence of cyanobacteria causes serious problems. Reversal of eutrophication, the general cause of the problem, is not always possible or may take long. A new method for fast and selective suppression of cyanobacteria was based on the original finding that cyanobacteria are much more sensitive to hydrogen peroxide than other phyto-plankton species (Drabkova *et al.*, 2007). The mechanism behind is suggested to relate to the observation that A-type flavoproteins are essential for photoreduction of oxygen and that this enzymatic routing produces H<sub>2</sub>O (water) in cyanobacteria (Helman *et al.* 2003), in marked contrast to the formation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in algae and plants. Using this concept, we arrived at the idea that in the ecosystem cyanobacteria are likely less auto-exposed to hydrogen peroxide than green algae, and may invest less in the expression of enzymes that protect against hydrogen peroxide. Accordingly, we proposed that application of *dilute and homogeneously dispersed* hydrogen peroxide directly into the water column of a lake will kill cyanobacteria and leave other vital biological functions ideally unaffected. The method has been tested and proved effective in a *Planktothrix aghardii* invaded recreation lake in the Netherlands (Matthijs *et al.*, 2012). Our present work focusses on the suitability of the method for suppression of *Microcystis* blooms. Questions posed relate to 1) effectiveness for different strains of *Microcystis*; 2) conditional expression of anti-oxidative stress enzymes; 3) getting inside into the induction of resistance or provoked changes in strain biodiversity; 4) interrogation of the potential role of microcystin as anti-oxidative stress factor (Zilliges *et al.*, 2011); 5) optimization of the method for field applications in *Microcystis* troubled lakes.

[1] Drabkova, M., Admiraal, W. and Marsalek, B. (2007). Combined exposure to hydrogen peroxide and light: selective effects on cyanobacteria, green algae and diatoms. *Environment. Sc. Technol.* 41, 309-314; [2] Helman, Y., Tchernov, D., Reinhold, L., Shibatea, M., Ogawa, T., Schwarz, R., Ohad, I., Kaplan, A (2003). Genes encoding A-type flavoproteins are essential for photoreduction of O<sub>2</sub> in cyanobacteria. *Current Biol.* 13, 230-235; [3] Matthijs, H.C.P., Visser, P.M., Reeze, B., Meeuse, J., Slot, P.C., Wijn, G., Talens, R., Huisman, J. (2012) Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. *Water Res.* 46, 1460-1472; [4] Zilliges, Y., Kehr, J.C., Meissner, S., Ishida, K., Mikkat, S., Hagemann, S., Kaplan, A., Boerner, T., Dittmann, E. (2011). The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. *PLoS ONE* 6, e17615.

## OC20: Our challenges for economic photobiological production of H<sub>2</sub> by cyanobacteria

**Hidehiro Sakurai<sup>1</sup>, Masaharu Kitashima<sup>2</sup>, Hajime Masukawa<sup>1,3</sup>, and Kazuhito Inoue<sup>1,2</sup>**

<sup>1</sup>Institute for Photobiological Hydrogen Production, Kanagawa University, Hiratsuka, Japan; <sup>2</sup>Department of Biological Sciences, Kanagawa University, Hiratsuka, Japan; <sup>3</sup>PRESTO, Japan Science and Technology Agency (JST), Kawaguchi, Saitama, Japan

We are proposing large-scale H<sub>2</sub> production on the sea surface utilizing nitrogen-fixing cyanobacteria [1]. Photobiological production of H<sub>2</sub> by cyanobacteria is considered to be one of the strong candidates for renewable energy because both the source of energy (Sun light) and the electron donor (water) are large in quantity. Nitrogenase catalyzes nitrogen fixation accompanied by uni-directional H<sub>2</sub> production, and in the absence of N<sub>2</sub>, all the electrons are allocated to H<sub>2</sub> production. By eliminating the uptake hydrogenase (Hup) activity through genetic engineering, nitrogen-fixing cyanobacteria can accumulate H<sub>2</sub> for a long time in the presence of O<sub>2</sub> evolved by photosynthesis. We have created more than 60 mutants ( $\Delta$ Hup, etc) from *Nostoc* sp. The *Nostoc* sp. PCC 7422  $\Delta$ Hup mutant accumulated H<sub>2</sub> to 20-30% (v/v) in 3 to 8 days, and the efficiency of light energy conversion into hydrogen was 3.7% vs visible light (about 1.7% vs. total solar radiation). In order that microalgae can be used for energy production, it is essential to reduce the energy costs of nutrients (N, P, K etc.) and CO<sub>2</sub> [2]. Although cyanobacteria require nutrients in the growth stage, they can be managed to produce H<sub>2</sub> for a long time without renewing the culture medium. Moreover, combined nitrogen can be omitted from the culture medium in the growth stage for nitrogen fixing cyanobacteria. In the H<sub>2</sub> production stage, CO<sub>2</sub> is absorbed for the synthesis of saccharides, but released during degradation of the latter as the electron donor for H<sub>2</sub> production (recycling of CO<sub>2</sub>). By optimizing the gas compositions in the growth stage and in the H<sub>2</sub> production stage, we have shown that *Nostoc* sp. PCC 7422  $\Delta$ Hup cells were able to produce H<sub>2</sub> for more than 60 days without changing the culture medium. In order that fuels derived from algae are economically viable, it was pointed out that the cost of bioreactors should be within several US\$ per m<sup>2</sup> [3]. We have shown that flexible transparent H<sub>2</sub>-barrier plastic bags (Wakhy bags) can be used as bioreactors for photobiological H<sub>2</sub> production. Our calculation of the applicability of H<sub>2</sub> production in the H<sub>2</sub>-barrier plastic bags in the future indicated that the leakage of H<sub>2</sub> over 60 days would be less than 1% of the H<sub>2</sub> produced [4]. One of our next important challenges is to increase the energy conversion efficiency (from solar to H<sub>2</sub>) under outdoor conditions.

[1] H. Sakurai, H. Masukawa (2007) *Mar. Biotechnol.* 9: 128-145; [2] Clarens *et al.* (2010) *Env. Sci. Technol.* 44: 1813; [3] Amos (2004) ([www.nrel.gov/docs/fy04osti/35593.pdf](http://www.nrel.gov/docs/fy04osti/35593.pdf)); [4] M. Kitashima, H. Masukawa, H. Sakurai, K. Inoue (2012) *Biosci. Biochem. Biotechnol.* 76: 831-833

## OC21: Assembly and export of extracellular polymeric substances (EPS) in cyanobacteria: a phylogenomic approach

Sara B. Pereira<sup>1</sup>, Rita Mota<sup>1</sup>, Catarina L. Santos<sup>1</sup>, Roberto De Philippis<sup>2</sup>, and Paula Tamagnini<sup>1,3</sup>

<sup>1</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; <sup>2</sup>Department of Agricultural Biotechnology, University of Florence, Florence, Italy; <sup>3</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

Many cyanobacteria produce extracellular polymeric substances (EPS), mainly composed of polysaccharides that can remain associated to the cell or be released into the surrounding environment (RPS-released polysaccharides). The particular characteristics of these EPS, such as the presence of two different uronic acids, sulphate groups and high number of different monosaccharides (up to 13), makes them very promising for biotechnological applications<sup>1,2</sup>. Despite the increasing interest in these polymers, the information about their biosynthetic pathways is still limited. Studies performed in other bacteria revealed that the mechanisms of EPS assembly and export are relatively conserved, generally following the Wzy-dependent or the ABC-dependent pathways, which require the involvement of polysaccharide copolymerase (PCP) and outer membrane polysaccharide export (OPX) proteins<sup>3,4</sup>. Our previous studies revealed that, in cyanobacteria, the genes encoding the proteins involved in EPS assembly and export (EPS-related genes) occur in multiple copies, scattered throughout the genome, either isolated or in small clusters<sup>1</sup>. Moving from this *in silico* analysis, the *tsp* of 7 EPS-related genes of *Cyanothece* sp. CCY 0110 were identified by 5'RACE. In addition, the regions upstream of the *tsp* were analyzed using the RegPredict software, revealing putative binding sites for the following transcriptional regulators: *ArsR* (*wzy2* and *wzc1*), *PerR* (*wzb1*) and *Crp* (*wzc1*).

To increase our knowledge on the genetics of cyanobacterial EPS assembly and export, 24 cyanobacterial genome sequences (belonging to strains from different orders/subsections, displaying distinct morphologies, and isolated from various ecological niches) were screened for the conserved domains typically associated to well-characterized systems of EPS production. In addition, phylogenetic trees were computed for the putative PCP and OPX identified using the Maximum-Likelihood and the Neighbour-Joining algorithms. Overall, these phylogenetic analyses unveiled a complex evolutionary history of the PCP and OPX proteins in cyanobacteria, characterized by specific losses, HGT events and numerous paralogous duplications. The data gathered here provides a first insight on the phylogenetic history of the EPS-related genes, and constitutes a robust basis for subsequent studies aiming to optimize EPS production in cyanobacteria.

[1] Pereira *et al* (2009) FEMS Microbiol Rev 33:917-941 [2] De Philippis *et al* (1998) Appl Environ Microbiol 64:1130-1132 [3] Cuthbertson *et al* (2009) Microbiol Mol Biol Rev 73:155-177 [4] Whitfield and Larue (2008) Nat Struct Mol Biol 15:121-123.

## OC22: Secondary Metabolism of the Cyanobacterium *Oscillatoria* PCC 6506: from Genome Sequencing to Cyanotoxin and Metabolite Biosyntheses

Olivier Ploux<sup>1</sup>, Annick Méjean<sup>1,2</sup>, Stéphane Mann<sup>1</sup>, Rabbia Mazmouz<sup>1,2</sup>

<sup>1</sup>UMR CNRS 7223, ENSCP, ChimieParisTech, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France; <sup>2</sup>Université Paris Diderot, 75013 Paris, France

Cyanobacteria produce a wide range of secondary metabolites including potent toxins for animals and humans [1]. Cases of animal death, due to cyanobacterial toxin exposure, are regularly reported. For instance, anatoxin-a and homoanatoxin-a, two cyanobacterial neurotoxins provoke the rapid death of animals by acute asphyxia, when ingested, because these alkaloids are potent agonists of the nicotinic acetylcholine receptor [2].

We have sequenced the genome of *Oscillatoria* PCC 6506 which produces anatoxin-a and homoanatoxin-a and identified at least seven clusters of genes likely implicated in the biosynthesis of secondary metabolites, either polyketides, non-ribosomal peptides, hybrids or ribosomal peptides [3].

We have identified the *ana* cluster of genes that is responsible for the biosynthesis of anatoxin-a and homoanatoxin-a [4]. Feeding experiments with labeled precursors followed by spectroscopic analyses and *in vitro* reconstitution of the first steps [5,6] gave the clue to the biosynthesis, tracked for a long time, of these major neurotoxins.

We have also identified the *cyr* cluster of gene responsible for the biosynthesis of cylindrospermopsin, a cytotoxin and showed that *Oscillatoria* PCC 6506 and other *Oscillatoria* strains produce, cylindrospermopsin and 7-epi-cylindrospermopsin [7]. We found that the last step of the biosynthesis of these toxins is catalyzed by a 2-oxoglutarate-dependent iron oxygenase, which stereospecificity varies from strain to strain [8].

The characterization of the other clusters of genes involved in the secondary metabolism of *Oscillatoria* PCC 6506 are under investigation, and so far we have identified a cluster responsible for the production of new cyanobactins, cyclic ribosomal peptides, and a cluster responsible for the biosynthesis of a putative siderophore.

[1] van Apeldoorn, M. E., van Egmond, H. P., Speijers, G. J., and Bakker, G. J. (2007) Mol. Nutr. Food Res. 51, 7-60; [2] Cadel-Six, S., Peyraud-Thomas, C., Brient, L., Tandeau de Marsac, N., Rippka, R., and Méjean, A. (2007) Appl. Environ. Microbiol. 73,7605-7614; [3] Méjean, A., Mazmouz, R., Mann, S., Calteau, A., Médigue, C., and Ploux, O. (2010) J. Bacteriol. 192, 5264-5265; [4] Méjean, A., Mann, S., Maldiney, T., Vassiliadis, G., Lequin, O., and Ploux, O. (2009) J. Am. Chem. Soc. 131, 7512-7513; [5] Méjean, A., Mann, S., Vassiliadis, G., Lombard, B., Loew, D., Ploux, O. (2010) Biochemistry 49, 103-113; [6] Mann, S., Lombard, B., Loew, D., Méjean, A., Ploux, O. (2011) Biochemistry 50, 7184-7197; [7] Mazmouz, R., Chapuis-Hugon, F., Mann, S., Pichon, V., Méjean, A., and Ploux, O. (2010) Appl. Environ. Microbiol. 76, 4943-4949; [8] Mazmouz, R., Chapuis-Hugon, S., Pichon, V., Méjean, A., and Ploux, O. (2011) ChemBioChem 12, 858-862.

## OC23: Biotechnological utility of inducible cyanobacterial promoters: biosensors for arsenic and heavy metals

Peter B. Kós<sup>1</sup>, Barbara Blasi<sup>1,2</sup>, Loredana Peca<sup>1,3</sup> and Imre Vass<sup>1</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, H-6726 Szeged, Hungary; <sup>2</sup>Department of Ecological and Biological Sciences, University of Tuscia, Largo San Camillo De Lellis, 01100, Viterbo, Italy; <sup>3</sup>National Institute of Research and Development for Biological Sciences, Institute of Biological Research Cluj-Napoca, 400015 Romania

The primary mode of regulation of stress responses and metabolic processes in prokaryotes is the alteration of expression levels of respective genes. This feature enables us to take benefits of the inducibility of promoters and use them in scientific study of genes as well as for biotechnological applications. Among the plethora of external stimuli that may result in changes of gene expression pattern of the organism in question, one need to select those that are specific to a limited and well characterized set of promoters and are not accompanied by adverse side effects with respect to the cell metabolism or homeostasis. As a first set of our systematic search we investigated the alteration in gene expression pattern caused by various inorganic ions that are toxic in higher concentrations, and selected the suitable promoters in *Synechocystis* PCC6803, a cyanobacterium [1].

We found that Zn<sup>2+</sup>, Ni<sup>2+</sup>, Co<sup>2+</sup>, AsO<sub>4</sub><sup>3-</sup>, and Cd<sup>2+</sup> ions caused significant specific activation of the respective promoters of *Synechocystis* sp. without concomitant unspecific stress responses in certain concentration ranges, while unspecific gene expressions could also be observed at high concentrations [2]. This finding facilitates establishment of experimental conditions for selective expression of genes to be studied or utilized.

The promoters of the chosen stress genes were fused with luxAB genes coding for bacterial luciferase and the constructs were introduced into the host genome. The resulting strains show luminescence when exposed to the respective ions. Beyond being experimental proof of the expected functioning of the promoters, the concentration dependent nature of the luminescent signal allows the utilisation of the constructed mutants as whole cell biosensors. While some of these constructs [3] are selective and sensitive enough for practical application, the sensitivity of the arsenic sensor is significantly lower than would be needed for demonstrating health hazard in environmental samples without further improvement. We also found that Zn<sup>2+</sup> inhibits the Ni<sup>2+</sup> dependent luminescent signal, indicating that the functioning of the luciferase system itself is also influenced by some of these ions. Nevertheless, this technology would enable establishment of biosensor strains selective of arbitrarily chosen set of environmental stimuli.

[1] Peca, L., P. B. Kós, and I. Vass. 2007. Characterization of the activity of heavy metal-responsive promoters in the cyanobacterium *Synechocystis* PCC 6803. *Acta Biol. Hung.* 58: 11-22; [2] Blasi, B., L. Peca, I. Vass and P. B. Kós Characterization of Stress Responses of Heavy Metal and Metalloid Inducible Promoters in *Synechocystis* PCC6803. *J. Microbiol. Biotechnol.* (2012), 22(2), 166-169; [3] Peca, L., P. B. Kós, Z. Mate, A. Farsang, and I. Vass. 2008. Construction of bioluminescent cyanobacterial reporter strains for detection of nickel, cobalt and zinc. *FEMS Microbiol. Lett.* 289: 258-264.

## OC24: Transcriptional activation by NtcA in the absence of consensus NtcA-binding sites in and *Anabaena* sp. PCC 7120 devB promoter

Sergio Camargo, Ana Valladares, Enrique Flores and Antonia Herrero

Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Américo Vespucio 49, E-41092 Sevilla, Spain, e-mail: avalla@ibvf.csic.es, sergio.camargo@ibvf.csic.es

In *Anabaena* sp. strain PCC 7120, differentiation of heterocysts takes place in response to the external cue of combined nitrogen deprivation. NtcA, a global transcriptional regulator of cyanobacteria, is required for the activation of expression of multiple genes involved in heterocyst differentiation including the differentiation-specific factor HetR and the regulatory protein PipX. The *pipX* gene is expressed in the differentiating cells, at intermediate to late stages of the process and contributes to normal heterocyst function. *In vitro*, PipX has a positive effect on transcription from some canonical NtcA-activated promoters and represents a booster for the expression of late heterocyst genes in *Anabaena*. To gain insight into the mechanism of specific transcription activation during the differentiation of heterocysts, the devBCA operon from *Anabaena* was analyzed. devBCA is expressed from two different promoters activated upon N step-down. The distal devB promoter (transcription start point (TSP) located at position -704) represents a canonical class II NtcA-activated promoter, including a consensus NtcA-binding site centered 39.5 nucleotides upstream from the TSP. Transcription activation from a second TSP (-454) requires NtcA and is impaired in *hetR* mutants. In a wild-type background, three different DNA fragments, including both or each individual promoter, directed *gfp* expression localized mainly to proheterocysts and heterocysts. Expression was undetectable in an *ntcA* background and, for the fragment including the proximal promoter alone, in a *hetR* background. In spite of the absence of consensus NtcA-binding sequences between the two TSPs, NtcA was shown to interact with this DNA region, and NtcA and its effector, 2-oxoglutarate, were necessary and sufficient for *in vitro* transcription from the -454 TSP. No HetR binding to the DNA or *in vitro* transcription from the proximal devB TSP promoted by HetR alone was detected. However, a moderate positive effect of HetR on NtcA binding to the DNA between the two TSPs was observed. Thus, the proximal devB promoter appears to represent a suboptimal NtcA-activated promoter for which HetR may act as a coactivator, with the physiological effect of restricting gene activation to conditions of prevalence of high NtcA and HetR levels, such as those taking place during heterocyst differentiation.

[1] Valladares A, Rodríguez V, Camargo S, Martínez-Noël G M A, Herrero A and Luque I. Specific role of the cyanobacterial PipX factor in the heterocysts of *Anabaena* sp. strain PCC 7120. *J Bacteriol* (2011) 193: 1172-1182; [2] Camargo S, Valladares A, Flores E and Herrero A. Transcription activation by NtcA in the absence of consensus NtcA-binding sites in an *Anabaena* heterocyst differentiation gene promoter. *J Bacteriol* (2012) doi: 10.1128/JB.05994-11

## OC25: Electrochemical characterization of the bidirectional hydrogenase of *Synechocystis* sp. PCC 6803

Chelsea McIntosh<sup>1</sup>, Frauke Germer<sup>2</sup>, Rüdiger Schulz<sup>2</sup>, Anne K. Jones<sup>1</sup>, Jens Appel<sup>2,3</sup>

<sup>1</sup>Department of Chemistry and Biochemistry and Center for Bioenergy and Photosynthesis, Arizona State University, Tempe, Arizona 85287, USA;

<sup>2</sup>Botanisches Institut, Universität Kiel, Am Botanischen Garten 1-9, D-24118 Kiel, Germany; <sup>3</sup>School of Life Sciences, Arizona State University, Tempe, Arizona 85287, USA

The cyanobacterium *Synechocystis* sp. PCC 6803 contains a single enzyme involved in hydrogen metabolism, the pentameric bidirectional hydrogenase (HoxEFUYH). One of the subunits (HoxF) of the enzyme was affinity tagged in *Synechocystis* and used to purify the whole enzyme complex to apparent homogeneity. Like the oxygen tolerant hydrogenases the active site of this type of enzyme cycles between only four different redox states and lacks the Ni-A EPR signal in the fully oxidized state [1].

Protein film electrochemistry was used to investigate its catalytic properties [2]. By the addition of ambient concentrations of oxygen the hydrogenase is inactivated promptly. At an oxygen concentration of 1 % 25 to 50 % of the maximal rate of the enzyme remain while reducing protons to hydrogen whereas when oxidizing hydrogen the enzyme is completely inhibited under these conditions. The inactive state of the enzyme is quickly reactivated upon removal of oxygen.

Whereas most previously characterized [NiFe]-hydrogenases seem to be preferential hydrogen oxidizing catalysts, the cyanobacterial enzyme works effectively in both directions with a bias to proton reduction. These unusual catalytic capabilities including the rapid reactivation are well suited for cyanobacteria that switch between oxygen production in the light and dark anoxic conditions.

[1] Germer F, Zebger I, Saggiu M, Lenzian F, Schulz R, Appel J (2009) Overexpression, isolation, and spectroscopic characterization of the bidirectional [NiFe] hydrogenase from *Synechocystis* sp. PCC 6803. *J Biol Chem* 284, 36462-36472; [2] McIntosh CL, Germer F, Schulz R, Appel J, Jones AK (2011) The NiFe-hydrogenase of the cyanobacterium *Synechocystis* sp. PCC 6803 is working bidirectional with a bias to H<sub>2</sub> production. *J Am Chem Soc* 133, 11308-11319

## OC26: A novel transporter required for biogenesis of *cbb3*-type cytochrome c oxidase in *Rhodobacter capsulatus*

Seda Ekici<sup>1</sup>, Honghui Yang<sup>2</sup>, Hans-Georg Koch<sup>3</sup>, Fevzi Daldal<sup>1\*</sup>

<sup>1</sup>Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104; <sup>2</sup>Present address: State Key Laboratory of Multiphase Flow in Power Engineering, Xi'an Jiaotong University, Xi'an 710049 China; <sup>3</sup>Institut für Biochemie und Molekularbiologie, Albert-Ludwigs-Universität Freiburg, D-79104 Freiburg, Germany

Acquisition, delivery and incorporation of metals to their respective metalloproteins are important cellular processes. These processes are tightly controlled so that cells are not exposed to free metal concentrations that would lead to harmful oxidative damages. Copper (Cu) is one such metal that is required as a cofactor in a variety of proteins. However, when present in excessive amounts, Cu is toxic due to its oxidative capability (1). Cytochrome c oxidases (Cox) are among the metalloproteins whose assembly and activity involves incorporation of Cu cofactor into their main catalytic subunits (2). In this study, we focused on the acquisition of Cu<sup>2+</sup> by the facultative phototroph *Rhodobacter capsulatus* for its incorporation into the heme-Cu binuclear center of its *cbb3*-type Cox (*cbb3*-Cox). Genetic screens identified a *cbb3*-Cox defective mutant that requires Cu<sup>2+</sup> supplement to produce an active *cbb3*-Cox. Complementation of this mutant using wild-type genomic libraries unveiled a novel gene (*ccoA*) required for *cbb3*-Cox biogenesis in *R. capsulatus* (3). In the absence of CcoA, cellular content of Cu decreases, and *cbb3*-Cox assembly and activity becomes defective. CcoA shows pronounced homology to Major Facilitator Superfamily (MFS) type transporter proteins (3). Members of this family are known to transport small solutes or drugs, but so far, no MFS protein was implicated into *cbb3*-Cox biogenesis (4). Remarkably, *ccoA*-deletion mutants revert very frequently to produce an active *cbb3*-Cox, bypassing the need for this transporter. Concomitantly, these revertants also become very sensitive to Cu<sup>2+</sup>, and their cellular Cu content reaches wild type-like concentrations (3). We conclude that CcoA affects intracellular Cu content and is required for *cbb3*-Cox biogenesis, providing novel insights on the maturation and assembly of membrane-integral metalloproteins, and on hitherto unknown function(s) of MFS type transporters in bacterial Cu acquisition.

[1] Banci, L. et al., (2010) *Cell. Mol. Life Sci.* 67, 2563-2589; [2] Ekici S., et al., (2011) *Biochem. Biophys Acta*, 1808, 937-946; [3] Ekici, S., et al., (2012) *mBio*, 3, issue 1 e00293-11; [4] Saier, M. H., et al., (2006) *Nucleic Acids Res.* 34, D274-D278.



## OC27: Photosensors and light response/adaptation in *Nostoc punctiforme*

**Daniela Ferreira, Elsie Campbell, and John C. Meeks**

Department of Microbiology, University of California, Davis, California, USA

All photosynthetic organisms tend to tune their metabolism in response to the quantity and quality of light environment. Such organisms have photosensory proteins to measure the light environment and report their findings through biochemical signal transduction pathways. Phytochromes are widespread photoswitchable red/far-red photosensors, with a conserved photosensory core binding to bilin, that function as key regulators of development and photosynthesis in higher plants. Cyanobacteria are known to possess phytochrome homologues and phytochrome-related proteins that also have bilin-binding GAF domains. However, the knowledge on cyanobacterial phytochromes is scarce and despite the extensive characterization of the phytochrome Cph1 from *Synechocystis*, thus far its *in vivo* function is unknown. We are currently studying the mechanisms used by the filamentous N<sub>2</sub>-fixing *Nostoc punctiforme* ATCC 29133 to sense light and transmit that information to the cell. Construction of *N. punctiforme* mutants defective in putative key photoreceptors and transcriptional analysis of cells grown under different light conditions were used to evaluate the response to changes in light in this cyanobacterium.

In *N. punctiforme*, the gene encoding NpCph1 (a phytochrome homologous to *Synechocystis* Cph1), NpF0020, is located upstream the gene encoding its putative response regulator, (NpCph1) and another ORF that encodes a protein carrying response regulator and histidine kinase domains. The three genes are probably transcribed as a single operon, driven by a promoter 165 bp upstream the NpF0020 start codon but the existence of a terminator downstream NpF0021 cannot be excluded. A mutation in NpF0020 was constructed and the analysis of the mutant phenotype in terms of growth, pigmentation and light/dark response revealed no significant differences between this strain and the wild-type. Our results also show that NpCph1 is not involved in the phototaxis of hormogonia nor in the response of *N. punctiforme* to high light (100 μmol m<sup>-2</sup> s<sup>-1</sup>). On the other hand, microarray data shows that, in *N. punctiforme*, the adaptation to high light involves an increase in the transcription of the operon encoding products essential for scytonemin biosynthesis (a cyanobacterial "sunscreen" induced upon exposure to UVA radiation) but surprisingly no changes were found for the transcription of genes encoding other cyanobacterial pigments (e. g. carotenoids, biliproteins, or chlorophyll *a*).

## OC28: Altered carbohydrate metabolism in glycogen synthase mutants of *Synechococcus* sp. strain PCC 7002 grown under hypersaline, nitrogen limitation and auto-fermentation conditions

**Guerra, L.T.<sup>1,3</sup>; Xu, Y.<sup>2</sup>; Li, Z.<sup>2</sup>; Ludwig, M.<sup>2</sup>; Bryant, D.A.<sup>2,4</sup>; Dismukes, G.C.<sup>1</sup>**

<sup>1</sup>Waksman Institute of Microbiology, Rutgers, the State University of New Jersey; <sup>2</sup>Department of Biochemistry and Molecular Biology, The Pennsylvania State University; <sup>3</sup>Chemistry Department, Princeton University; <sup>4</sup>Department of Chemistry and Biochemistry, Montana State University

Glycogen is the major carbon and energy reserve in the cyanobacterium *Synechococcus* sp. PCC 7002 and the biosynthesis of osmolytes like sucrose and glucosylglycer(-ol/-ate) (GG) competes with glycogen for precursors. In an attempt to increase production of soluble sugars, we constructed mutant strains in which both glycogen synthase genes (*glgA-I* and *glgA-II*) or the glucose-ADP pyrophosphorylase gene (*glgC*) were deleted by cassette mutagenesis. We subjected these strains to nitrogen limitation or high salt, which are conditions known to further increase the amounts of intracellular carbohydrates (1, 2). The mutants grew under photoautotrophic conditions only slightly slower than the WT and were incapable of synthesizing glycogen under any condition. Under standard growth conditions (0.3 M NaCl) the amount of soluble sugars accumulated by the  $\Delta glgA-I \Delta glgA-II$  double mutant was 2.2-fold higher than the WT, while the increase in the  $\Delta glgC$  mutant was less pronounced. After 24 h of N-limitation WT cells accumulated 4.5-fold more glycogen concomitantly with substantial pigment degradation, whereas the mutants did not accumulate large amounts of any carbohydrate and retained higher pigment contents. The  $\Delta glgC$  mutant was unable to grow in medium containing 1 M NaCl, demonstrating the importance of GG as an osmoprotectant. Under the same conditions the  $\Delta glgA-I \Delta glgA-II$  mutant accumulated 3-fold more sucrose and 1.7-fold more GG than the WT. Moreover, large amounts (29 mol/10<sup>17</sup> cells of sucrose and 298 mol/10<sup>17</sup> cells of GG) were also detected in the growth medium, which indicated that the  $\Delta glgA-I \Delta glgA-II$  mutant was capable of secreting soluble sugars into the medium spontaneously without the need for additional transporters. Significantly, an additional 462 mol/10<sup>17</sup> cells of total soluble sugars could be extracted from this strain by hypo-osmotic shock with no detectable loss in cell viability and with no effect on the impermeability of cellular membranes. Transmission electron microscopy analysis showed evidence of vesicle formation for the  $\Delta glgA-I \Delta glgA-II$  strain grown in 1M NaCl. Although the contents of the observed vesicles are unknown, we postulate they are used to excrete sugars. During auto-fermentation in the dark the  $\Delta glgA-I \Delta glgA-II$  mutant had a similar catabolic rate to the WT and catabolized the smaller pool of insoluble sugars instead of the soluble sugars. In contrast, the  $\Delta glgC$  mutant had a very limited pool of insoluble sugars and had a 4.5-fold decrease in catabolic rate. These observations suggest that these specific soluble sugars are not preferred substrates for fermentation and are rather used solely as osmolytes. The  $\Delta glgA-I \Delta glgA-II$  mutant serves as a biological cell factory for excretion of sugars produced from photosynthetically fixed CO<sub>2</sub>, and the identification of strains with increased insoluble sugars accumulation capacity is ideal for increasing auto-fermentative rate and increase yields of biofuel precursors.

[1] Hagemann, M. 2011. Molecular biology of cyanobacterial salt acclimation. *FEMS Microbiology Reviews* 35:87-123; [2] Stevens, S. E., D. L. Balkwill, and D. A. M. Paone. 1981. The effects of nitrogen limitation on the ultrastructure of the cyanobacterium *Agmenellum quadruplicatum*. *Archives of Microbiology* 130:204-212.

**OC29: Transcription profiling of *Synechococcus* sp. PCC 7002 by RNAseq****Marcus Ludwig<sup>1</sup>, Chyue Yie Chew<sup>1</sup>, and Donald A. Bryant<sup>1,2</sup>**<sup>1</sup>Dept. of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802 USA; <sup>2</sup>Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717 USA

The cyanobacterium *Synechococcus* sp. PCC 7002 grows well under euryhaline conditions, tolerates very high irradiation levels, and has one of the shortest known doubling times. Furthermore, it can be easily manipulated genetically, and a system for complementation of mutations and overproduction of proteins is available, making this strain an excellent platform for biotechnological applications. Cyanobacteria must acclimate continuously to changes of environmental conditions, such as irradiation levels, nutrient supply and changes of temperature and salinity. Adjustments can be made on different levels: gene expression, protein maturation, assembly and stability, and through post-translational modifications of enzymes.

In this study we investigated the impact of transcriptional regulators on the global transcriptome of *Synechococcus* 7002. Some regulators in cyanobacteria have more general functions (e.g., sigma factors) and others are more specific. Deletion mutants were constructed for several regulators, and the global transcription patterns of the resulting mutant strains were analyzed via SOLiD™ sequencing of cDNA. Three genes in *Synechococcus* 7002 are annotated as *fur* (ferric uptake regulator) genes; two of these could be deleted. The deletion of the third *fur* gene, however, was not possible. Global transcriptome analysis revealed that the regulators act as transcription repressors and that one controls a set of genes including a zinc ABC transporter and that the second is involved in an oxidative stress response. The third *fur* gene, for which the mutant and wild-type alleles could not be fully segregated, regulates iron uptake.

A transcription activator that is active under micro-oxic conditions was identified. Its regulon comprises a single gene cluster, *acsF2-ho2-hemN-desF*, that encodes oxygen-dependent enzymes involved in chlorophyll, biliverdin and heme biosynthesis and a fatty acid desaturase. Global transcription profiling suggested that the [O<sub>2</sub>] was the only factor affecting expression level of these genes [1]. Promoter fusions to a *yfp* gene were constructed and introduced into *Synechococcus* 7002, and YFP synthesis was controlled by oxygen level.

The data obtained in this study revealed regulatory networks at the level of transcriptional regulation for *Synechococcus* 7002. Comparisons of transcriptomes for more than 30 different growth conditions and/or specific treatments of cells showed that several regulation patterns represent general stress responses among cyanobacteria, whereas other responses are specific for *Synechococcus* 7002 [1, 2]. These data provide valuable information for identifying appropriate promoters for various applications, and they allow one to identify genes of interest in well-characterized regulons, both of which are important for future metabolic engineering for biotechnological applications.

[1] Ludwig and Bryant, 2011. *Front Microbiol.* 2:41; [2] Ludwig and Bryant, 2012. *Front Microbiol.* 3:145**OC30: Assembly and characterization of Oxygen Consuming Devices (OCDs): the laccase-based device****Catarina C Pacheco<sup>1</sup>, Zsófia Büttel<sup>1</sup>, Filipe Pinto<sup>1,2</sup> and Paula Tamagnini<sup>1,2</sup>**<sup>1</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; <sup>2</sup>Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal

The BioModularH<sub>2</sub> project (FP6, NEST-2005-Path-SYN) aimed at preparing a photoautotrophic chassis to accommodate constructed standardized parts and devices, primarily designed for hydrogen production. The organism chosen to be used as a chassis was the unicellular *Synechocystis* sp. PCC 6803 since it is one of the most studied cyanobacteria and there are plenty of molecular tools for its manipulation. The preparation of the chassis started with the generation of a *Synechocystis* deletion mutant lacking an active bidirectional hydrogenase (*ΔhoxYH*), since a heterologous hydrogenase was going to be introduced. The *ΔhoxYH* mutant was characterized at different levels (physiological, proteomics and transcriptional) revealing that it is robust and can be used as a photoautotrophic chassis for the integration of synthetic devices [1]. Additionally, *Synechocystis* genome was screened for putative neutral sites, for the integration of the synthetic devices without impairing the chassis viability/fitness (oral communication by F Pinto *et al.*).

In parallel, to achieve the low intracellular level of oxygen required for an optimal hydrogenase activity (O<sub>2</sub> mainly generated by photosynthesis), several synthetic OCDs were constructed based on proteins native and heterologous to *Synechocystis*. These devices are one- or two-protein modules designed to be compatible with the BioBrick™ system and the codon usage was optimized both for *Escherichia coli* and *Synechocystis*. The OCDs reduce O<sub>2</sub> directly to H<sub>2</sub>O (one-protein modules) or to H<sub>2</sub>O<sub>2</sub> (two-protein modules), and in the latter case a catalase is coupled to dismutate the H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O. Appropriate regulation of the devices was achieved using constitutive or inducible promoters, and in this case, the F2620 BioBrick (based on luxR promoter) was used.

The OCDs performance was first assessed in *E. coli* evaluating protein expression, specific enzymatic activities and oxygen consumption. A laccase-based device (one-protein module) was fully characterized and revealed to be functional in *E. coli*. Moreover, other OCDs based on laccase variants obtained through directed evolution (Weizmann Institute) were also assembled and characterized. The synthetic OCDs with best performance will be introduced and tested in our photoautotrophic chassis – *Synechocystis* sp. PCC 6803.

[1] Pinto F, van Elburg KA, Pacheco CC, Lopo M, Noirel J, Montagud A, Urchueguía JF, Wright PC and Tamagnini P (2012) Construction of a chassis for hydrogen production: physiological and molecular characterization of a *Synechocystis* sp. PCC 6803 mutant lacking a functional bidirectional hydrogenase. *Microbiology* 158:448-464.

### OC31: Analysis of an inactive biosynthetic gene cluster leads to discovery of new cyanobactins pircyclamides in strains of *Microcystis*

**Niina Leikoski, David P. Fewer, Jouni J. Jokela, Matti Wahlsten, Pirita Alakoski and Kaarina Sivonen**

Division of Microbiology, Department of Food and Environmental Sciences, University of Helsinki, Finland

Cyanobactins are highly diverse cyclic peptides found in many cyanobacteria. They are assembled through the cleavage and modification of short precursor proteins. An inactive cyanobactin gene cluster has been described from the genome of *Microcystis aeruginosa* NIES843<sup>1</sup>. Here we report the discovery of functional cyanobactin gene clusters in strains of the genus *Microcystis* guided by this silent cyanobactin gene cluster. The end-products of the gene clusters were structurally diverse cyclic peptides, which we named pircyclamides. Some of the pircyclamides consisted solely of proteinogenic amino acids while others contained disulfide bridges and some were prenylated or geranylated. The pircyclamide gene clusters encoded between 1 and 4 precursor genes. They encoded highly diverse core peptides ranging in length from 5-17 amino acids with just a single conserved amino acid. Heterologous expression of the pir gene cluster from *Microcystis aeruginosa* PCC7005 in *Escherichia coli* confirmed that this gene cluster is responsible for the biosynthesis of pircyclamides. Chemical analysis demonstrated that *Microcystis* strains could produce an array of pircyclamides some of which are geranylated or prenylated. The genetic diversity of pircyclamides in a bloom sample was explored and 19 different pircyclamide precursor genes were found. This study provides evidence for a stunning array of pircyclamides in *Microcystis*, a worldwide occurring bloom forming cyanobacteria.

[1] Donia MS and Schmidt EW 2011 Linking chemistry and genetics in the growing cyanobactin natural products family. *Chemistry and biology* 18:508-519. (4)

### OC32: Production of hydrogen by transgenic strains of the cyanobacterium *Anabaena variabilis* expressing hydrogenase genes

**Fatthy M. Morsy<sup>1,2</sup>, and Teresa Thiel<sup>1</sup>**

<sup>1</sup>Department of Biology, University of Missouri-St. Louis, St. Louis, MO, USA; <sup>2</sup>Botany Department, Faculty of Science, Assiut University, Egypt

The cyanobacterium *Anabaena variabilis* is a filamentous, photosynthetic prokaryote that is capable of growth with nothing more than sunlight, air and water with small amounts of inorganic compounds. Unlike most other photosynthetic organisms, this strain can fix atmospheric dinitrogen to ammonium in a process called nitrogen fixation and it does so under aerobic growth conditions. This is because the oxygen-labile enzyme, nitrogenase, is protected in specialized cells called heterocysts that provide a nearly anaerobic environment for nitrogen fixation. While nitrogenase is capable of hydrogen production itself, it is not an efficient hydrogenase. Therefore, we attempted to express true Fe-hydrogenase genes in *A. variabilis* with the goal of producing large amounts of hydrogen using sunlight as the only source of energy. The sources of the *hydA* genes and their maturation factor genes were *Clostridium acetobutylicum* and *Chlamydomonas reinhardtii*. The *hydA* genes, with or without their maturation factor genes, were fused to the ferredoxin-NADP<sup>+</sup> reductase (FNR) gene. This includes the two promoters along with the N-terminal CpcD-like domain of FNR for overexpressing Fe-hydrogenase in heterocysts as well as vegetative cells. FNR catalyzes the electron transfer from reduced ferredoxin to NADP<sup>+</sup> in the terminal step of the photosynthetic electron transport chain, while the CpcD-like domain targets FNR to the photosynthetic membranes. Fusing the Fe-hydrogenase genes to the CpcD-like domain should target the Fe-hydrogenase to the photosynthetic apparatus, replacing the normal FNR and facilitating the transfer of electrons from ferredoxin directly to the Fe-hydrogenase. We report here the construction of these transgenic strains and their production of hydrogen.

### OC33: Glycosylated mycosporine-like amino acids from the terrestrial cyanobacterium *Nostoc commune*

**Ehsan Nazifi<sup>1</sup>, Kei Matsui<sup>2</sup>, Minami Yamaba<sup>2</sup>, Chieri Tsuji<sup>2</sup>, Naoki Wada<sup>3</sup>, Seiichi Matsugo<sup>3</sup>, and Toshio Sakamoto<sup>1,2,3,\*</sup>**

<sup>1</sup>Division of Life Science, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan; <sup>2</sup>Division of Biological Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan; <sup>3</sup>School of Natural System, College of Science and Engineering, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan; \*E-mail: tsakamot@staff.kanazawa-u.ac.jp

Mycosporine-like amino acids (MAAs) are water-soluble pigments that absorb UV radiation of 280 to 340 nm, and structurally distinct MAAs are known in taxonomically diverse organisms. In the terrestrial cyanobacterium *Nostoc commune*, a structurally unique MAA covalently bound to oligosaccharides has been reported [1], and the glycosylated structure of this MAA is thought to allow for the interaction with extracellular matrices. The hydrophobic pigment scytonemin absorbs UV-A radiation of 320 to 400 nm and occurs exclusively in the cyanobacterial sheath. With both the UV-absorbing pigments of MAAs and scytonemin in its extracellular matrices, *N. commune* is thought to be able to adapt to terrestrial environments with high levels of solar radiation. In this study, novel MAAs were purified from the cyanobacterium *Nostoc commune*, and their chemical structures were characterized. An MAA with an absorption maximum at 335 nm was identified as a pentose-bound porphyrin-334 derivative with a molecular mass of 478 Da. Another identified MAA had double absorption maxima at 312 and 340 nm and a molecular mass of 1050 Da. Its unique structure consisted of two distinct chromophores of 3-aminocyclohexen-1-one and 1,3-diaminocyclohexen and two pentose and hexose sugars. The third glycosylated MAA had an absorption maximum at 322 nm and a molecular mass of 612 Da. The 1050-Da MAA had strong radical scavenging activity *in vitro* and contributed approximately 27% of the total radical scavenging activities in a water extract of *N. commune* [2]. Moreover, the purified scytonemin quenched an organic radical *in vitro* and accounted for up to 10% of the total activity of an ethanol extract of *N. commune* [3]. These results suggest that these glycosylated MAAs and scytonemin have multiple roles as a UV protectant and an antioxidant relevant to anhydrobiosis in *N. commune*.

[1] Böhm, G.A., Pfeleiderer, W., Böger, P. and Scherer, S. (1995) *J. Biol. Chem.* 270:8536-8539; [2] Matsui, K., Nazifi, E., Kunita, S., Wada, N., Matsugo, S. and Sakamoto, T. (2011) *J. Photochem. Photobiol. B* 105:81-89; [3] Matsui, K., Nazifi, E., Hirai, Y., Wada, N., Matsugo, S. and Sakamoto, T. (2012) *J. Gen. App. Microbiol.* in press

## OC34: Carbohydrate loading of cyanobacteria for biofuel fermentation

**Klaus Benedikt Möllers<sup>1</sup>, Thomas Grochalski<sup>1</sup>, Jacob H. Jacobsen<sup>1</sup>, David Cannella<sup>2</sup>, Henning Jørgensen<sup>2</sup>, Niels-Ulrik Frigaard<sup>1</sup>**

<sup>1</sup>Department of Biology, University of Copenhagen, Helsingør, Denmark; <sup>2</sup>Forest and Landscape, University of Copenhagen, Frederiksberg, Denmark

Cyanobacteria are photosynthetic microbes that prosper under a wide range of environmental conditions. Their sturdiness is based on the successful combination of effective metabolic pathways that hold great promise for use in sustainable production of biofuels and other bioproducts [1]. Due to its rapid doubling time (~4 hours), its ease of genetic manipulation, and accumulation of different storage compounds (glycogen and cyanophycin), the unicellular cyanobacterium *Synechococcus* sp. PCC 7002 represents a robust research organism [2,3,4,5,6]. Thus, metabolic engineering of this organism can be used as a powerful tool to produce renewable energy resources and valuable metabolic products. The aim of this study is to increase the total carbohydrate content of *Synechococcus* cells and to use this biomass for biofuel fermentation. This will be done by various approaches including nutrient limitation and genetic modification of *Synechococcus* followed by enzymatic treatment and subsequent fermentation of the sugar-enriched cyanobacterial biomass to ethanol by an industrial production strain (Thermosacc) of the yeast *Saccharomyces cerevisiae*. *Synechococcus* cells were grown under standard conditions (38°C, 250  $\mu\text{E m}^{-2} \text{s}^{-1}$ , 1% v/v  $\text{CO}_2$ ) in A+ medium containing different concentrations of sodium nitrate or potassium phosphate. A total sugar content of 46% w/w of the dry weight was obtained when *Synechococcus* cells were grown under nitrogen limitation (0.24 mg/ml  $\text{NaNO}_3$ ) for 48 hours to an optical density ( $\text{OD}_{730}$ ) of 4.6 compared to standard conditions (1 mg/ml  $\text{NaNO}_3$ ) that led to a total sugar content of 14% w/w at an  $\text{OD}_{730}$  of 6.9. Lysis of *Synechococcus* cells were carried out using a treatment with cell-wall-degrading lysozyme at 37°C followed by a treatment with polyglucose-degrading alpha-amylases at 60–85°C. Following the enzymatic hydrolysis the produced glucose was fermented by *S. cerevisiae*. The conversion yield of total glucose in the cyanobacterial biomass to ethanol was 70–90%. These results show that cyanobacterial biomass can be efficiently used as feedstock for microbial bioethanol fermentation.

[1] Vermaas FJ (2001) Photosynthesis and Respiration in Cyanobacteria. Encyclopedia of Life Sciences, Wiley; [2] Smith AJ (1982) Modes of Cyanobacterial Carbon Metabolism. In: Carr NG and Whitton BA (ed.) The Biology of Cyanobacteria, pp. 59-63. University of California Press; [3] Allen MM (1984) Cyanobacterial Cell Inclusions. Ann. Rev. Microbiol. 38: 1-25; [4] Ernst A and Böger P (1985) Accumulation of Glycogen and the Induction of Nitrogenase Activity in *Anabaena variabilis*. Journal of General Microbiology 131: 3147-31 53; [5] Victor H. Kollman (1979) Photosynthetic Characterization and Preparation of labelled Carbohydrates in *Agmenellum quadruplicatum*. Carbohydrate Research, 73: 193-202; [6] Cohen Y and Gurevitz M (2006) The Cyanobacteria- Ecology, Physiology and Molecular Genetics. Prokaryotes 4: 1074–1098

## OC35: Accumulation of extracellular carbohydrates in a scytonemin-deficient mutant of *Nostoc punctiforme* exposed to UVA stress

**Tanya Soule**

Department of Biology, Indiana University-Purdue University Fort Wayne, Indiana, 46805, USA

As phototrophs, cyanobacteria are often found in environments of high UV irradiance in order to capture the solar energy needed for photosynthesis. As such, one of their many strategies for coping with UV stress is to augment their extracellular matrix with polysaccharides and sunscreens. In a high UV irradiance environment, the accumulation of extracellular polysaccharides (EPS) can protect the cells from desiccation and act as an osmoprotectant while sunscreens function by preventing damaging photons from reaching potential cellular targets or from generating reactive oxygen species. In particular, the sunscreen scytonemin is deposited as a yellow-brown pigment in the extracellular sheath of many cyanobacteria where it intercepts harmful radiation in the UVA range (320-400 nm). While studies using field cultures of *Nostoc commune* exposed to UVA did not show an increase in EPS production [1], the relationship between EPS and scytonemin production has not been established. Using a scytonemin-deficient mutant of the cyanobacterium *Nostoc punctiforme* ATCC 29133 (referred to herein as SCY 59) compared to the wild type, cultures of each strain were exposed to either white light or white light supplemented with UVA in triplicate for 6 days. Upon harvesting, the cultures were washed and sonicated to release extracellular carbohydrates. The total extractable and ethanol-precipitable (higher molecular weight) extracellular carbohydrates were quantified using the colorimetric Phenol-Sulfuric Acid (PSA) assay [2]. Preliminary results indicate that SCY 59 accumulated significantly more extracellular carbohydrates when exposed to UVA compared to either strain under white light only and the wild type exposed to UVA, all of which had similar levels of extracellular carbohydrate production. Since the absence of scytonemin results in an increased UVA influx to exposed cells, it appears that SCY 59 is responding to this stress by accumulating EPS. Additional EPS not only increases the path length for incident UVA but also reduces free radical access to cellular targets and may serve as a matrix for UV-protective carotenoids. Since the mutation in SCY 59 is localized to the scytonemin operon, and EPS production is not inhibited in this strain, it appears as though scytonemin and EPS production are not coupled but rather respond to UVA stress in an independent manner. Future studies will focus on further examining the relationship between EPS and scytonemin production.

[1] Ehling-Schulz et al. (1997) J. Bacteriol. 179:1940; [2] Dubois et al. (1956) Anal. Chem. 28:350



## OC36: SigG is an ECF sigma factor required for normal differentiation and stress survival of *Nostoc punctiforme*

Nicole Cassel<sup>1</sup>, Hugo Medina<sup>1</sup>, Karen LeGrand<sup>2</sup>, and Michael L. Summers<sup>1</sup>

<sup>1</sup>Department of Biology, California State University, Northridge, CA, USA; <sup>2</sup>Food Science and Technology Department, University of California, Davis, CA, USA

Akinetes are spore-like cells resistant to desiccation and cold that differentiate from vegetative cells of the filamentous cyanobacterium *N. punctiforme* in response to phosphate or potassium limitation and low light. Previous microarray data has identified a putative extracytoplasmic function (ECF) sigma factor, NpF4153 (SigG), and an adjacent downstream anti-sigma factor, NpF4154, as potential genes involved in akinete induction in *N. punctiforme*. GFP transcriptional reporter strains confirmed the array results, showing increased expression from the *sigG* promoter in akinetes, heterocysts, and following stresses imposed by heat or EDTA. The *zwf* model system of akinete induction [1] was used to unveil a potential regulon for SigG by two separate microarray analyses comparing the *zwf/sigG* double mutant relative to a *zwf* strain under both vegetative growth conditions and during akinete formation. Genes showing decreased expression in the double mutant included outer membrane proteins, lipid biosynthesis proteins, proteases, as well as many hypothetical proteins. Transcriptional start sites of genes shown to be down-regulated due to deletion of *sigG* in both microarrays were determined by random amplification of cDNA ends, and a putative SigG consensus sequence was determined. To determine interaction between SigG and the N-terminal end of the NpF4154 anti-sigma factor, a GST pull down assay was performed. The N-terminal predicted cytoplasmic domain of the anti-sigma fused to GST was able to pull-down SigG-His6 from an *E. coli* crude lysate, indicating a specific interaction between SigG and F4154N-GST. A strain expressing a SigG-GFP protein fusion was shown by confocal laser microscopy to have GFP-fluorescence localized to the periphery of the cell under normal growth conditions. Such protein localization was lost following exposure to short periods of heat, indicating release of sigma factor from the cell membrane following stress. Survival studies comparing wild-type and  $\Delta sigG$  mutant strains following exposure to heat or outer membrane disruption using EDTA showed reduced survival for the mutant strain. The mutant also exhibited a significant delay in heterocyst formation following nitrogen starvation relative to the wild-type strain. Experiments to identify genes under direct control of SigG using an *in vitro* run-off microarray transcription assay will be presented. Together these data supports the hypothesis that NpF4153-4154 encode proteins that act in a manner that is typical of an ECF sigma / anti-sigma factor pair that is important for normal expression of genes involved in cell differentiation and envelope biosynthesis/repair.

[1] Argueta, C and M.L. Summers. 2005. Characterization of a model system for the study of *Nostoc punctiforme* akinetes. Archives of Microbiology 183:338-346.

## OC37: Dynamic formation of protein assembly centers in stressed cyanobacteria

Samantha J. Bryan<sup>1</sup>, Nigel J. Burroughs<sup>2</sup>, Dmitriy Shevela<sup>3</sup>, Jianfeng Yu<sup>4</sup>, Lu-Ning Liu<sup>1</sup>, Quan Xue<sup>5,6</sup>, Isabel Llorente-Garcia<sup>5,6</sup>, Dirk Schneider<sup>7</sup>, Mark C. Leake<sup>6</sup>, Lutz A. Eichacker<sup>3</sup>, Peter J. Nixon<sup>4</sup> & Conrad W. Mullineaux<sup>1</sup>

<sup>1</sup>School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK; <sup>2</sup>Mathematics Institute and Warwick Systems Biology Centre, University of Warwick, Coventry CV4 7AL, UK; <sup>3</sup>Department of Mathematics and Natural Science, University of Stavanger, 4036 Stavanger, Norway; <sup>4</sup>Department of Life Sciences, Imperial College London, London SW7 2AZ, UK; <sup>5</sup>Oxford Centre for Integrative Systems Biology (OCISB), Dept of Biochemistry, University of Oxford, Oxford OX1 3QU, UK; <sup>6</sup>Clarendon Laboratory, Department of Physics, University of Oxford, Parks Road, Oxford OX1 3PU, UK and Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK; <sup>7</sup>Institut für Pharmazie und Biochimie, Johannes Gutenberg-Universität Mainz, D-55128 Mainz, Germany

The Vipp1 protein is essential in cyanobacteria and plants for the generation of a normal thylakoid membrane architecture and efficient photosynthesis (1,2). However, its mode of action remains elusive (3). Using fluorescent protein tagging and live-cell imaging we demonstrate that Vipp1 undergoes dramatic relocalisation under high light, with similar effects under other stress conditions. Under normal conditions, Vipp1 is predominantly dispersed in the cytoplasm. However, within minutes of exposure to high light, Vipp1 coalesces into puncta with net relocation to the vicinity of the cytoplasmic and thylakoid membranes. Utilizing pull-downs and mass spectrometry we identified a distinct protein cargo associated with Vipp1 under high light, including photosynthetic and stress-related proteins, translation and protein assembly factors. Our data suggest that Vipp1 is central to the formation of stress-induced localized protein assembly centers, enabling enhanced protein synthesis and delivery to membranes. The related PspA protein could play a similar role throughout the bacterial kingdom.

[1] Kroll, D. et al. VIPP1, a nuclear gene of *Arabidopsis thaliana* essential for thylakoid membrane formation. Proc. Natl Acad. Sci USA 98, 4238-4242 (2001); [2] Westphal, S., Heins, L., Soll, J. & Voithknecht, U.C. Vipp1 deletion mutant of *Synechocystis*: a connection between bacterial phage shock and thylakoid biogenesis? Proc. Natl Acad. Sci USA 98, 4243-4248 (2001); [3] Voithknecht, U.C., Otters, S., Hennig, R. & Schneider, D. Vipp1: a very important protein in plastids?! J. Exp. Bot. 63, 1699-1712 (2012).

## OC38: Evolutionary origin of photorespiration: Phylogenetic and biochemical studies of core cycle enzymes

Martin Hagemann, Ramona Kern, Hermann Bauwe

Universität Rostock, Institut für Biowissenschaften, Abteilung Pflanzenphysiologie, Albert-Einstein-Str. 3, D-18059 Rostock, Germany; martin.hagemann@uni-rostock.de

The photorespiratory pathway is essential for organisms performing oxygenic photosynthesis, cyanobacteria, algae and plants, in the present day O<sub>2</sub>-containing atmosphere. The presence of a plant-like 2-phosphoglycolate cycle in cyanobacteria indicated that not only genes of oxygenic photosynthesis but also genes encoding photorespiratory enzymes were endosymbiotically conveyed from ancient cyanobacteria to eukaryotic oxygenic phototrophs. BlastP analyses with plant photorespiratory proteins identified several proteins in cyanobacteria and algae with surprisingly high sequence similarities. To verify that not only the sequence but also the biochemical activity of these enzymes is conserved, selected proteins from the cyanobacteria *Synechocystis* PCC 6803, *Anabaena* PCC 7120 and *Cyanothece* sp. PCC 7822, as well as from the algae *Chlamydomonas reinhardtii* and *Cyanidioschyzon merolae* were overexpressed in *E. coli* and biochemical analyzed.

As an example, we present data on our recent analyses of hydroxypyruvate reductase (HPR). Among cyanobacteria, we found three clusters of putative HPR proteins. The HPR1-like protein of *Cyanothece* sp. PCC 7822 showed the closest sequence and biochemical similarities to the AtHPR1. Additional HPR-like proteins were found in other cyanobacterial strains. The cyanobacterial AII8087, which clusters also closely to the plant HPR1 is characterized by a very low  $K_m$  value for hydroxypyruvate, whereas the SII1556 protein had the highest  $V_{max}$  with hydroxypyruvate. However, only the cyanobacterial enzyme Slr2123 preferred hydroxypyruvate over glyoxylate as was found for the AtHPR1. Furthermore, 3-phosphoglycerate dehydrogenases from *Synechocystis* and *Anabaena* showed also sequence similarities to HPR, but likely take part in the phosphoserine pathway of cyanobacteria.

## OC39: Nitrite transport activity of the ABC-type cyanate transporter of the cyanobacterium *Synechococcus elongatus* PCC 7942

**Shin-ichi Maeda and Tatsuo Omata**

Department of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

The fresh water cyanobacterium *Synechococcus elongatus* strain PCC 7942 has the ATP-binding cassette transporter for nitrate and nitrite, which is encoded by the *nrtABCD* genes and has high affinity for both substrates ( $K_m \sim 1 \mu\text{M}$ ). The *nrtABCD* genes form the *nirA* operon (*nirA-nrtABCD-narB*), in which *nirA* and *narB* are the structural genes for nitrite reductase and nitrate reductase, respectively. The NA3 mutant of *S. elongatus*, which was constructed by deleting the *nrtABCD* genes from the *nirA* operon, was defective in active transport of nitrate. However, it retained significant uptake activity of nitrite. Detailed analysis of the nitrite uptake activity by the NA3 mutant revealed that the cyanobacterium has an active transport system for nitrite (NIT), which has an apparent  $K_m$  ( $\text{NO}_2^-$ ) of  $20 \mu\text{M}$  [1].

In this work, we used a nitrogen-responsive promoter-reporter fusion to isolate a mutant defective in the NIT activity. We found that the NIT activity depends on the *cynABD* genes, encoding an ATP-binding cassette transporter previously identified as a cyanate (NCO<sup>-</sup>) transporter [2]. The NIT activity was competitively inhibited by cyanate with a  $K_i$  (NCO<sup>-</sup>) value of  $0.025 \mu\text{M}$ , showing that the *cynABD* genes encode a bispecific cyanate/nitrite transporter with a three-orders-of-magnitude higher affinity for cyanate than for nitrite. The *CynABD* transporter was induced under the conditions of nitrogen deficiency, and the induced cells showed a  $V_{max}$  value of 11-13  $\mu\text{mol}$  per mg of chlorophyll per hour for nitrite or cyanate, which could supply  $\sim 30\%$  of the amount of nitrogen required for optimum growth and only 5-10% of the cellular capacity of cyanate decomposition as previously determined in the presence of high concentrations (0.5-2 mM) of cyanate in medium [2, 3]. On the basis of its kinetic properties and distribution among cyanobacteria, the physiological role of this transporter in nitrogen metabolism of cyanobacteria is discussed.

[1] Maeda, S., Okamura M., Kobayashi M, and Omata T. 1998, Nitrite-specific active transport system of the cyanobacterium *Synechococcus* sp. strain PCC7942. *J. Bacteriol.* 180:6761-6763; [2] Espie, G. S., Jalali F., Tong T., Zacal N. J., and So A. K. 2007, Involvement of the *cynABDS* operon and the  $\text{CO}_2$ -concentrating mechanism in the light-dependent transport and metabolism of cyanate by cyanobacteria. *J. Bacteriol.* 189:1013-1024; [3] Miller, A. G., and Espie G. S. 1994, Photosynthetic metabolism of cyanate by the cyanobacterium *Synechococcus* UTEX 625. *Arch. Microbiol.* 162:151-157.

## OC40: Role Of the two *hik31* operons in regulating carbohydrate and photosynthetic metabolism in *Synechocystis* sp. PCC 6803

**Sowmya Nagarajan, Louis A. Sherman**

Purdue University, Department of Biological Sciences, West Lafayette, IN. 47907 USA

Signal transduction proteins sense environmental changes and regulate the versatile metabolism of the model cyanobacterium *Synechocystis* sp. PCC 6803 that can be used to produce biofuels. We are interested in duplicated signal transduction operons each containing a histidine kinase (*hik31*), response regulator, and hypothetical protein, on both the chromosome (C3) and plasmid pSYSX (P3). We believe that the hypothetical protein is involved in activating the other two genes, making this a true three-component system (3CS) and we have made a detailed study of these duplicated regulatory operons. Our **overall goal** is to determine the functions of both operons, their relationship to each other, as well as their impact on central metabolism and industrial relevance.

Methodology used included construction of deletion mutants, measurements of growth in different conditions, ultrastructural analysis, microarrays and RT-PCR to monitor gene transcription, and next generation sequencing to determine plasmid copy number and genotype of our Wild-Type (WT) lab strain. We will emphasize the microarray results performed on the C3 and P3 deletion mutants compared to WT under mixotrophic conditions. These experiments enable us to determine the regulatory networks and the targets of each signal transduction system. Our results suggest that the two copies are not redundant and are involved in regulating **separate and common** metabolic events: C3 is involved in negative control of autotrophic growth in the light, whereas P3 is involved in positive control of heterotrophic growth in the dark. We conclude that both operons are differentially and temporally regulated, that C3 is the primarily expressed copy, and that P3 acts as a back-up to maintain appropriate gene dosages in high demand conditions. Both operons share an integrated regulatory relationship, are induced in high light, glucose and in active cell growth. The re-sequencing results define the genotype of our WT strain and shed light on the importance of plasmid genes in complementing the functions of the chromosomal genes. We believe that P3 is an important regulator that controls metabolism at night and in addition, influences photosynthesis and cell division.

Therefore, this **master regulatory system** regulates basic photosynthetic and carbohydrate metabolism, as well as cell division through processes in the light and the dark, and is important to the physiology of the cell. The generation of faster growing mutants, inducible promoters for metabolic engineering in our well characterized wild-type lab strain, intracellular inclusions storing carbon and nitrogen, as well as a secreted pigment are of interest from the viewpoint of industrial microbiological processes. Our results reveal new connections between gene regulation in the diurnal cycle of this organism and carbon processing metabolic pathways and metal transport, and have implications for improved growth for biofuel production.

## OC41: Mapping and characterization of *Synechocystis* sp. PCC 6803 neutral sites for the integration of synthetic devices

Filipe Pinto<sup>1,2</sup>, Catarina C. Pacheco<sup>1</sup> and Paula Tamagnini<sup>1,2</sup>

<sup>1</sup>IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; <sup>2</sup>Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal

Cyanobacteria are photosynthetic prokaryotes that are promising “low-cost” microbial cell factories due to their simple nutritional requirements, metabolic plasticity, and molecular tools for their genetic manipulation are available. The unicellular non-nitrogen fixing *Synechocystis* sp. PCC 6803 is the best studied cyanobacterial strain and its genome was the first to be sequenced. The vast amount of physiological and molecular data available, together with a relatively small genome, makes *Synechocystis* suitable for computational metabolic modeling and to be used as a photoautotrophic chassis [1,2]. Synthetic biology applications require the accommodation of several synthetic devices into the chassis, and consequently the identification of genomic sites where devices can be inserted without impairing cell viability or fitness. In order to identify these so-called genomic “neutral sites”, an *in silico* analysis on *Synechocystis*’ genome was performed, and 16 putative open reading frames (ORFs) were selected as targets. After a preliminary analysis three ORFs were discarded based on the genomic context, and a transcription analysis of the remaining was performed leading to the selection of the five loci exhibiting minor or no expression. Moreover, to assess the neutrality of these five sites, integrative vectors were constructed to generate disruption mutants, which were characterized in terms of viability/fitness. The vectors include a BioBrick™ compatible interface insulated by transcriptional terminators allowing the insertion of synthetic devices into a specific neutral site. A reporter gene (*gfp*), with or without a constitutive promoter, was cloned in each vector that was subsequently used to transform *Synechocystis*. The integration of *gfp* in the chromosome was confirmed by molecular techniques, and confocal microscopy revealed GFP fluorescence only when the reporter is under the control of a constitutive promoter, pointing out to the robustness of the interface (no leakiness from the chromosomal backbone).

This work provides a set of new BioBrick™ compatible vectors for the integration of synthetic devices into five chromosomal neutral sites of a photoautotrophic chassis based on *Synechocystis* sp. PCC 6803. In addition, *Synechocystis* mutants (chassis) ready to receive purpose-built synthetic modules/circuits will also be available.

[1] Montagud A, Zeleznik A, Navarro E, de Córdoba PF, Urchueguía JF and Patil KR (2011) Flux coupling and transcriptional regulation within the metabolic network of the photosynthetic bacterium *Synechocystis* sp. PCC6803. *Biotechnol J* 6:330-42; [2] Pinto F, van Elburg KA, Pacheco CC, Lopo M, Noirel J, Montagud A, Urchueguía JF, Wright PC and Tamagnini P (2012) Construction of a chassis for hydrogen production: physiological and molecular characterization of a *Synechocystis* sp. PCC 6803 mutant lacking a functional bidirectional hydrogenase. *Microbiology* 158:448-64.

## OC42: Newly discovered chlorophyll (chlorophyll *f*) and its function in oxygenic photosynthetic organism

Min Chen\*, Yaqiong Li, Yuankui Lin

School of Biological Sciences, University of Sydney, New South Wales 2006, Australia

Chlorophylls are the essential pigments for photosynthesis. There are five forms of chlorophylls that have been discovered to date, chlorophyll *a*, *b*, *c*, *d* and *f*. Chlorophyll *a* plays a vital role in all oxygenic photosynthesis. Chlorophylls *b* and *c* can only serve as accessory photosynthetic pigments in antenna complexes.

The red-shifted chlorophylls (Chl *d* and Chl *f*) allow the photosynthetic organisms the critical advantage of using longer wavelength photons (690-760 nm) that are not absorbed by organisms containing chlorophyll *a*, *b*, *c*. Those red-shifted chlorophylls allow oxygenic photosynthesis to extend its physical limits, even further into the infrared region, which may open up associated bio-energy applications [1].

Recently, we have identified a new chlorophyll from stromatolites, chlorophyll *f* [2]. Chlorophyll *f* has a maximum Qy absorption peak at 706 nm and maximum fluorescence emission at 722 nm at room temperature in methanol, making it the most red-shifted chlorophyll. The molecular formula for chlorophyll *f* is C<sub>55</sub>H<sub>70</sub>O<sub>6</sub>N<sub>4</sub>Mg, and it has molecular weight of 906 Da. NMR analysis indicates a formyl group is located at the C-2 position, with the rest of the structure identical to Chl *a*, *i.e.* Chl *d* and Chl *f* have the similar chemical structure as Chl *a* except that a formyl group replacement: at C-3 in Chl *d* and at C-2 in Chl *f*. Such formyl group replacement in Chl *d* and in Chl *f* lead to their red-shifted spectral properties, which is different from formyl group substitution at C-7 in Chl *b* which causes a blue shift.

We have isolated and cultured a filamentous Chl *f*-containing cyanobacterium and have investigated the photophysiological properties. The 16s rRNA sequence of this newly-isolated cyanobacterium was completed and the further phylogenetic analysis was performed to reveal the phylogenetic relationship to other cyanobacteria. HPLC analysis on the Chl *f*-containing cyanobacterium confirmed that Chl *f*, as an accessory photopigment, maximally can reach up to 10-15% of total chlorophyll under 720-750 infrared LED light, with the balance being Chl *a*. The photosynthetic function of Chl *f* and its biophysical processing (such as the energy flow driven by Chl *f*) in the newly-isolated cyanobacterium will be discussed. The ecological distribution of the red-shifted chlorophylls will be also discussed.

[1] Chen M and Blankenship RB (2011) Expanding the solar spectrum used by photosynthesis, *Trends in Plant Science* 16:427-431; [2] Chen M, Schliep M, Willows R, Cai Z-L, Neilan BA, Scheer H (2010) A red-shifted chlorophyll, *Science* 329:1718-1719.



### OC43: The terminal emitter $L_{CM}$ (ApcE) as the primary site of non-photochemical quenching of the phycobilisome fluorescence emission by orange carotenoid protein in the cyanobacterium *Synechocystis* sp. PCC 6803

Igor N. Stadnichuk<sup>1</sup>, Mikhail F. Yanyushin<sup>2</sup>, Evgeny G. Maksimov<sup>3</sup>, Evgeny P. Lukashev<sup>3</sup>, Gábor Bernát<sup>4</sup>, Irina V. Elanskaya<sup>3</sup>, Pavel M. Krasilnikov<sup>3</sup>, and Vladimir Z. Paschenko<sup>3</sup>

<sup>1</sup>A.N. Bakh Institute of Biochemistry, Russian Academy of Sciences, Moscow, Russia; <sup>2</sup>Institute of Fundamental Problems of Biology, Russian Academy of Sciences, Puschino, Russia; <sup>3</sup>Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia; <sup>4</sup>Plant Biochemistry, Ruhr-University Bochum, Bochum, Germany

In cyanobacteria, the thermal dissipation of excess excitation energy (=non-photochemical fluorescence quenching) at the level of phycobilisome (PBS)-antennae is triggered by absorption of blue-green light by the photoactive orange carotenoid protein (OCP) [1]. According to a current report this mechanism is evolutionarily rather old [2]. An *in vitro* reconstituted system with OCP and PBS' isolated from the cyanobacterium *Synechocystis* sp. PCC 6803 provided direct evidence that the OCP is not only a photosensor in this process, but acts also as an effector [3,4]. To localize the primary site of quenching, we have analyzed the role of chromophorylated polypeptides of the PBS core using an *in vitro* system with isolated PBS components as well as PBS-deficient mutants [5]. The results have shown that neither bulk allophycocyanin trimers nor the ApcD or ApcF terminal emitters within the PBS are directly subjected to quenching. In contrast, the fluorescence emission of the core-membrane linker  $L_{CM}$  is effectively quenched by photo-activated OCP *in vitro*. The conclusion is further supported by concentration dependence and fluorescence lifetime-measurements, 77 K fluorescence emission and fluorescence excitation spectra studies of quenched PBS as well as with ApcE-less *Synechocystis* cells. The data suggest that besides its central role in excitation energy transfer and as a key structural element in the PBS,  $L_{CM}$  is the primary site of photoprotective excitation quenching in the PBS core. Changes in fluorescence lifetime of the fully assembled PBS and isolated  $L_{CM}$  and linear dependences of fluorescence intensity on OCP concentration indicate that the quenching of  $L_{CM}$  has a static character, while the corresponding secondary quenching of assembled PBS is of a dynamic type. A model for OCP-PBS interaction is proposed based on the 3D structures of the corresponding proteins. According to the model the OCP molecule is wedged between the chromophorylated PB domain of  $L_{CM}$  inside the PBS core and the surface of the thylakoid membrane and there could be a direct contact between the phycocyanobilin chromophore of LCM and the ketocarotenoid chromophore of OCP. The distance between these chromophores is estimated as about 15 Å and two-fold longer in the unquenched state respectively.

[1] Kirilovsky D., Adv. Exp. Med. Biol. (2010) 675, 139-159; [2] Bernát G. et al., Plant Cell Physiol. (2012) 53, 528-542; [3] Stadnichuk I.N. et al., Dokl. Biochem. Biophys. (2011), 439, 167-170; [4] Gwizdala M. et al., Plant Cell (2011) 23, 2631-2643; [5] Stadnichuk I.N. et al., Biochim. Biophys. Acta (2012) doi: 10.1016/j.bbabi.2012.03.02.

### OC44: Mobilization of RSF1010 between *Anabaena* strains illustrates conjugation among cyanobacteria

M. Pilar Garcillán-Barcia<sup>1</sup>, David Encinas<sup>1</sup>, Ana Valladares<sup>2</sup>, Enrique Flores<sup>2</sup>, Antonia Herrero<sup>2</sup>, Julien Guglielmini<sup>3</sup>, Eduardo Rocha<sup>3</sup> and Fernando de la Cruz<sup>1</sup>

<sup>1</sup>Departamento de Biología Molecular e Instituto de Biomedicina y Biotecnología de Cantabria (IBBTec), Universidad de Cantabria-CSIC-IDICAN, C. Herrera Oria s/n, E-39011 Santander, Spain; <sup>2</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Av. Américo Vespucio 49, E-41092 Seville, Spain; <sup>3</sup>Microbial Evolutionary Genomics group, Institut Pasteur, CNRS UMR3525, 25 rue Dr Roux, 75724 Paris, France.

Conjugation from *E. coli* has proven to be a suitable method for introducing DNA into several cyanobacteria [1]. However, rigorous evidence for plasmid conjugation between cyanobacteria was lacking. *In silico* analysis of cyanobacterial plasmids reveals the presence of a variety of genetic determinants for conjugation, suggesting potential conjugative ability in this bacterial phylum [2]. As a case in point, five out of the six native plasmids of *Anabaena* sp. strain PCC7120 contain apparently intact conjugation genetic determinants. RSF1010 is a broad-host range mobilizable plasmid that replicates in several cyanobacterial genera, including *Anabaena* [1]. We tested the ability of *Anabaena* sp. strain PCC7120 to mobilize a neomycin-resistant derivative of RSF1010 to a streptomycin-resistant derivative of *Anabaena* sp. strain PCC7118. Transconjugants containing the RSF1010 derivative showed no structural rearrangements, when analyzed by growth in selective media, PCR and restriction endonuclease analysis. In summary, our report highlights the ability of a proteobacterial plasmid to circulate among cyanobacteria by conjugation, suggesting a mechanism for the acquisition of proteobacterial-related genes found in this phylum.

[1] Koksharova OA, Wolk CP. (2002) Appl Microbiol Biotechnol. 58(2):123-37; [2] Guglielmini J, Quintais L, Garcillán-Barcia MP, de la Cruz F, Rocha EP. (2011) PLoS Genet. 7(8):e1002222.

## OC45: The auxiliary proteins of Photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803

**Julian J. Eaton-Rye<sup>1</sup>, Robert D. Fagerlund<sup>1</sup>, Simon A. Jackson<sup>1</sup>, Peter D. Mabbitt<sup>1</sup>, Mark G. Hinds<sup>2,3</sup> and Sigurd M. Wilbanks<sup>1</sup>**

<sup>1</sup>Department of Biochemistry, University of Otago, Dunedin 9054, New Zealand; <sup>2</sup>Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; <sup>3</sup>Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, Parkville 3010, Australia

The core of Photosystem II (PS II) is made up of two reaction center proteins, D1 (PsbA) and D2 (PsbD) and two chlorophyll *a*-binding antenna proteins, CP47 (PsbB) and CP43 (PsbC). These proteins have homologues in anoxygenic photosynthetic bacterial reaction centers; however, PS II has an increased complement of polypeptides. These proteins include ~12 low-molecular-weight (LMW) hydrophobic polypeptides that form a ring around the core and a "cap" of 3 or more hydrophilic polypeptides that cover the oxygen-evolving center (OEC). These auxiliary PS II proteins were acquired prior to endosymbiotic uptake of cyanobacteria. The crystal structure of cyanobacterial PS II has revealed that the cap of the OEC is composed of three polypeptides: PsbO, PsbU and PsbV, and two large loops from CP43 and CP47. In green algae and plants, PsbU and PsbV are absent but two different proteins, PsbP and PsbQ, are present. However, biochemical and genomic studies have now established that there are PsbP and PsbQ homologues in all photosynthetic lineages: and in cyanobacteria the corresponding homologues, CyanoP and CyanoQ, are lipidated at their N-termini. Successful water splitting by PS II carries with it the metabolic cost of light-induced photodamage (chiefly to D1) and the resulting need for a repair cycle to support ongoing O<sub>2</sub>-evolving activity. It is likely that a major role of the auxiliary proteins is to assist in water splitting and PS II repair. We have investigated the role of the LMW PsbT protein: removal of this subunit increased susceptibility to photodamage and accelerated the rate of incorporation of replacement D1. Moreover, in this mutant, electron transfer between Q<sub>A</sub> and Q<sub>B</sub>, the primary and secondary quinone electron acceptors of PS II, was slowed and the PSII-specific electron acceptor 2,5 dimethyl-*p*-benzoquinone blocked Q<sub>A</sub>-oxidation. These effects could be prevented, and in some cases reversed, by the addition of HCO<sub>3</sub><sup>-</sup>, a PS II-specific cofactor that binds to the non-heme iron between QA and QB. In addition, we have obtained the X-ray-derived structure of CyanoQ and the NMR structures of CyanoP and a third cyanobacterial lipoprotein, Psb27 (Psb27 is found in all photosynthetic lineages and is involved in the PS II repair cycle). Structural features of these polypeptides will be presented together with docking models that place these proteins adjacent to channels in the OEC cap that may be required to allow substrate H<sub>2</sub>O or cofactors to enter, and O<sub>2</sub> and protons to exit, to and from the Mn<sub>4</sub>O<sub>5</sub>Ca active site of the OEC.

## OC46: Control of the low inorganic carbon induced expression of the *flv4-2* operon in *Synechocystis* by the asRNA *As1\_flv4* and the transcriptional regulators *NdhR* and *Sll0822*

**Marion Eisenhut<sup>1</sup>, Jens Georg<sup>2</sup>, Stephan Klähn<sup>2</sup>, Isamu Sakurai<sup>3</sup>, Henna Silén<sup>3</sup>, Pengpeng Zhang<sup>3</sup>, Wolfgang R. Hess<sup>2</sup> and Eva-Mari Aro<sup>3</sup>**

<sup>1</sup>Department of Plant Biochemistry, University of Duesseldorf, Germany; <sup>2</sup>Faculty of Biology, University of Freiburg, Germany; <sup>3</sup>Department of Biochemistry and Food Science, Plant Biology, University of Turku, Finland

Cyanobacteria show strong transcriptional alterations in response to changes in inorganic carbon (C<sub>i</sub>) levels. In *Synechocystis flv4*, *sll0218* and *flv2*, organized in the *flv4-2* operon, belong to the most up-regulated genes after shift from high C<sub>i</sub> (3% CO<sub>2</sub> in air, HC) to low C<sub>i</sub> (ambient 0.038% CO<sub>2</sub> in air) conditions. We could demonstrate that the encoded proteins are important in photoprotection of photosystem II (PSII) under LC conditions [1]. The small membrane protein Sll0218 stabilizes the PSII dimer and enables the Flv2/Flv4 heterodimer to open up an alternative PSII electron valve [2].

Though ongoing progress the C<sub>i</sub> dependent regulatory network is still not fully understood. Recently, the *Synechocystis* genome was screened for the occurrence of non-coding RNAs (ncRNA). More than 1,000 *cis*-antisense RNAs (asRNA) and more than 300 possibly *trans*-acting ncRNAs could be identified [3]. Among those RNAs three asRNAs and one ncRNA associated with the *flv4-2* operon were detected. We investigated the regulatory impact of one of those asRNAs, designated as *As1\_flv4*, on the expression of the *flv4-2* operon [4]. When wild type cells were shifted from HC to LC conditions we observed an inversely correlated accumulation of *As1\_flv4* RNA and *flv4-2* operon mRNA. Artificial overexpression of *As1\_flv4* led to decreased *flv4-2* mRNA accumulation. Additional promoter activity analysis showed a transient induction of the *as1\_flv4* promoter after LC shift, which is likely negatively regulated by the AbrB-like transcriptional regulator Sll0822. In contrast, *NdhR* controls probably indirectly the transcription of the *flv4-2* operon. The results indicate that the asRNA *As1\_flv4* establishes a safety threshold to prevent unfavorable synthesis of the Flv2, Flv4 and Sll0218 proteins in the early phase after a change in C<sub>i</sub> levels [4].

A hypothetical model on the interplay of the asRNA and the transcriptional regulators to tightly control the C<sub>i</sub> dependent expression of the *flv4-2* operon will be discussed.

[1] Zhang P., Allahverdiyeva Y., Eisenhut M., Aro E.M. (2009) Flavodiiron proteins in oxygenic photosynthetic organisms: photoprotection of photo-system II by Flv2 and Flv4 in *Synechocystis* sp. PCC 6803. PLoS One 4:e5331; [2] Zhang P., Eisenhut M., Brandt A.M., Carmel D. Allahverdiyeva Y., Salminen T., Aro E.M. (2012) Operon *flv4-flv2* provides cyanobacteria with a novel photoprotection mechanism. Plant Cell accepted; [3] Mitschke, J., Georg, J., Scholz, I., Sharma, C.M., Dienst, D., Bantscheff, J., et al. (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC 6803. Proc Natl Acad Sci USA 108: 2124-2129; [4] Eisenhut M., Georg J., Klähn S., Sakurai I., Silén H., Zhang P., Hess W.R., Aro E.M. The antisense RNA *As1\_flv4* in the cyanobacterium *Synechocystis* sp. PCC 6803 prevents premature expression of the *flv4-2* operon upon shift in inorganic carbon supply. submitted

## OC47: The CcmI subunit of the CcmFHI heme ligation complex is an apocytochrome c chaperone and forms a ternary complex with CcmE and apocytochrome c<sub>2</sub>

**Andreia F. Verissimo<sup>1</sup>, Mohamad A. Mohtar<sup>1</sup>, Hongui Yang<sup>1,2</sup>, Xiaomin Wu<sup>1,2</sup>, Carsten Sanders<sup>1</sup> and Fevzi Daldal<sup>1</sup>**

<sup>1</sup>Biology Department, University of Pennsylvania, Philadelphia, USA; <sup>2</sup>Present Address: State Key Laboratory of Multiphase Flow in Power Engineering, Xi'an Jiaotong University, Xi'an, China

Cytochrome c plays a fundamental role during photosynthesis and respiration. *Rhodobacter* (*R.*) *capsulatus* is an anoxygenic nonsulfur phototrophic purple bacterium that contains a variety of c-type cytochromes. Cytochrome c<sub>2</sub> shuffles electrons from the cytochrome bc<sub>1</sub> complex to the reaction center during photosynthesis and to the cbb<sub>3</sub> cytochrome c oxidase during respiration. Cytochrome c maturation (Ccm) is the post-translational process responsible for covalent ligation of heme cofactor to the Cys residues at the conserved heme-binding site (CXXCH) within the apocytochromes. Holocytochromes formed by this process then assemble into the active electron transfer complexes. In *R. capsulatus* and other Gram-negative bacteria, ten membrane-bound proteins (CcmABCDEFGHI and CcdA) carry out the Ccm process (1). Of these proteins, CcmI is a bipartite protein formed of an N-terminal membrane-embedded domain with two transmembrane helices encompassing a cytoplasmic loop, and a large C-terminal periplasmic domain containing protein-protein interaction motifs (1). Together with CcmF and CcmH, CcmI forms a membrane-integral complex responsible for heme ligation to the apocytochromes (2). Using *R. capsulatus* apocytochrome c<sub>2</sub> as a Ccm substrate, we show that CcmI binds to apo-, but not holo-cytochrome c<sub>2</sub>, establishing its role as an apocytochrome c chaperone (3). Major physical interactions occur between the C-terminal portions of these two proteins. Conserved structural elements in apocytochrome c<sub>2</sub>, like the heme-binding site Cys or the heme iron axial ligands His and Met, are less important for these interactions (3). CcmE has a unique role during Ccm as it binds covalently to the heme via a His residue located at a conserved heme binding motif (HXXXXY), and delivers it to the apocytochrome c substrates (4). CcmE is a monotopic protein with a large periplasmic domain presenting a rigid β-barrel core structure with a flexible C-terminal portion (5). We found that CcmE interacts with both CcmI and apocytochrome c<sub>2</sub> to form a ternary complex, and that the membrane anchor of CcmE is not required for these interactions. In contrast to what was observed with CcmI, the heme-binding site Cys residues within apocytochrome c<sub>2</sub> seem to be critical for its interaction with CcmE.

[1] Sanders, C et al, (2010) Trends Microbiol. 18, 266-274; [2] Sanders, C. et al, (2008) JBC 283, 29715-22; [3] Verissimo, AF. et al (2011) JBC 286, 40452-63; [4] Kranz, R.G., et al (2009) Microbiol. Mol. Biol. Rev. 73, 510-528; [5] Thöny-Meyer, L (2003) Biochemistry 42, 13099-105

## OC48: The Respiratory Terminal Oxidases of Cyanobacteria

**Georg Schmetterer<sup>1</sup>, Ronald Stebegg<sup>1</sup>, Heinrich Burgstaller<sup>1</sup>, and Markus Mikulic<sup>1</sup>**

<sup>1</sup>Institute of Physical Chemistry, University of Vienna, Vienna, Austria

Cyanobacteria are bioenergetically unique: they are the only organisms, in which the 2 most important bioenergetic processes, oxygenic photosynthesis and aerobic respiration, occur in the same compartment. In darkness, all cyanobacteria respire with O<sub>2</sub> as the terminal electron acceptor. The Respiratory Terminal Oxidases (RTOs) are the key enzymes of cyanobacterial respiration, as they are the only respiratory electron transport components not directly involved in photosynthetic electron transport. The availability of more than 60 total genomic sequences from many different, but also closely related cyanobacterial strains has allowed us to define the existence of 7 different RTOs in cyanobacteria that belong to three protein families: 1) homologs of mitochondrial-type cytochrome c oxidase; 2) homologs of cytochrome bd quinol oxidase (well known from *Escherichia coli*); and 3) homologs of plastidic terminal oxidase (Ptox). The enzymes of protein family 1 can be further subdivided into 1a) genuine cytochrome c oxidase (Cox), similar to that of mitochondria, but with only 3 subunits; 1b) ARTOs, homologs of cytochrome c oxidase, but with characteristic sequence deviations; 1c) homologs of cbb<sub>3</sub>-type cytochrome c oxidase (well known from purple bacteria), but with only 2 (instead of 4-8) subunits present. The characteristics of the ARTOs concern short sequence deviations from Cox in subunits I and/or II: subunits I of Cox invariably contain the sequence WAHMF, the ARTO sequence at this position is slightly different; subunits II of Cox contain the Cu binding sequence CAELCGAYH, the ARTO sequence at this position is DSQFSGTYF (or very similar). The ARTO-type sequence characteristics can occur in both subunits I and II (ARTO type 1), only in subunit I (ARTO type 2) or only in subunit 2 (ARTO type 3). We will present a large table of all RTOs in all currently sequenced cyanobacteria. One striking result is: *Anabaena variabilis* ATCC29413 contains 10 (!) RTOs, a record for any organism known. Some general conclusions can be made from the table: a) all cyanobacteria contain at least one RTO, b) no RTO is present in all cyanobacteria, c) the set of RTOs present in a cyanobacterium varies widely from strain to strain, even among closely related strains. Our working postulate is: In every cyanobacterium that contains more than 1 RTO, each RTO must have a specific function. In a few cases, we were able to define this function. Homologs of cytochrome cbb3 are rare in cyanobacteria, but despite the presence of only the 2 largest polypeptides, we have recently shown that this protein is enzymatically an active cytochrome c oxidase in *Synechococcus* sp. PCC7942. In 3 cyanobacteria, *Synechocystis* sp. PCC6803, *Anabaena variabilis* ATCC29413, and *Anabaena* (*Nostoc*) sp. PCC7120 we were able to show that Cox is bioenergetically the most proficient RTO: Knock-out mutations of the cox locus led to mutant strains incapable of chemo-organo-heterotrophic growth.

## OC49: Effects of Carbonyl-Cyanide m-Chlorophenylhydrazone on the Induction of Intracytoplasmic Membrane Assembly in *Rhodobacter sphaeroides*

Kamil Woronowicz, Oluwatobi B. Olubanjo, Daniel Sha, Joseph M. Kay and Robert A. Niederman

Department of Molecular Biology and Biochemistry, Rutgers University, Busch Campus, 604 Allison Road, Piscataway, NJ 08854-8082, USA

The effect of carbonyl-cyanide m-chlorophenyl-hydrazone (CCCP) on the assembly of the intracytoplasmic membrane (ICM) was examined in *Rba. sphaeroides*. CCCP acts as a protonophore, which blocks generation of the electrochemical proton gradient necessary for membrane insertion of light-harvesting (LH) polypeptides. ICM formation was induced at low aeration in concentrated cell suspensions for 8 h and after a further 4 h, ICM vesicles (chromatophores) and an upper-pigmented band (UPB) containing membrane invagination sites were isolated by rate-zone sedimentation on sucrose density gradients. In order to determine whether partially assembled pigment-protein complexes can be isolated from CCCP-treated cells in association with participating assembly factors, isolated membrane fractions, along with those from untreated control cells, were subjected to clear-native electrophoresis to resolve their pigment-protein complexes for proteomic analysis. CCCP treatment resulted in blockage of membrane insertion of LH and reaction center (RC) polypeptides, shown by fluorescence induction/relaxation measurements to be reflected in a diminished quantum yield of primary charge separation, cessation in expansion of the functional absorption cross-section and a >4-fold slowing in the RC electron transfer turnover rate. Proteomic analysis of chromatophore gel bands after CCCP treatment showed a 2.7-fold reduction in spectral counts in the LH2 (bottom) band, while for the RC-LH1 (top) band and the F1FO-ATPase enriched upper intermediate band, 1.9- and 1.7-fold decreases, respectively, were observed together with a 1.7-fold diminution in pyridine nucleotide transhydrogenase counts. This provided further evidence for the role of the electrochemical proton gradient in membrane translocation of nascent LH apoproteins and extends these findings to the additional energy-transducing complexes. The higher value for LH2 is consistent with the accelerated rate of LH2 synthesis and assembly at this stage of the induction process, whereas the smaller decreases for the RC-LH1 cores, F1FO-ATPase and transhydrogenase reflect assembly rates at a basal level for these complexes. It is likely that the CCCP-induced decline in levels of antenna and energy transducing complexes is due to cytoplasmic degradation of unassembled apoproteins, since for 35 soluble enzymes, the ratio of 0.99 for spectral counts of control/treated proteins indicated that protein synthesis was unaffected by CCCP treatment. Importantly, for the UPB fraction, proteomic analysis showed that CCCP treatment resulted in accumulation of ~2-fold greater levels of the preprotein translocase SecY, the SecA translocation ATPase, Sec D and SecF insertion components, and chaperonins DnaJ and DnaK. This is consistent with the possibility that these general membrane assembly factors had accumulated in association with nascent LH and RC assembly intermediates.

Supported by U. S. DOE Grant No. DE-FG02-08ER15957

## OC50: *Rhodobacter capsulatus* AmtY is functional when expressed in *Escherichia coli*

Zahra Choolei, Pier-Luc Tremblay, and Patrick C. Hallenbeck

Département de microbiologie et immunologie, Université de Montréal, CP 6128, succursale Centre-ville, Montréal, PQ, H3C 3J7 Canada

Amt proteins are found in almost all forms of life; bacteria (Amt, ammonium transporter), yeast (Mep, methylamine permease protein) and animals Rh (rhesus factor) (1). AmtB is a homotrimeric cytoplasmic membrane protein with a hydrophobic pore. Conduction of external  $\text{NH}_4^+$  requires deprotonation at the exterior, transfer of  $\text{NH}_3$  through the cytoplasmic membrane, and reprotonation at the AmtB cytoplasmic surface. The role of AmtB in many bacteria is unclear since passive diffusion of  $\text{NH}_3$  permits growth on ammonium except at low pHs and very low ammonium concentrations. However, in some purple non-sulfur photosynthetic bacteria AmtB has been shown to be essential for ammonium switch-off of nitrogenase activity. In some bacteria AmtB is thought to regulate nitrogenase activity by the ammonium induced sequestration of GlnK and DraG to the membrane (3, 4). However, complex formation, at least in some AmtB variants, is not sufficient (4).

*Rhodobacter capsulatus* possesses two genes coding for ammonium transport proteins, *amtB* and *amtY*. A previous study showed that AmtB was solely responsible for nitrogenase regulation and an *amtY* mutant had no apparent phenotype, i.e. was normal for methylamine transport and nitrogenase switch-off. Thus, the function of AmtY was unclear. Here we show, by qPCR, that *amtY* is transcribed in *R. capsulatus* under N-limiting conditions, again raising questions as to its function. We cloned *amtY* and expressed it in an *amtB* *Escherichia coli* strain (GT1001). Surprisingly, AmtY appears functional in a number of respects in that species. When expressed in *E. coli* AmtY is active in methylammonium transport and can correct the growth defect on ammonium of the *amtB*-strain. However, although RcAmtY is capable of binding EcGlnK, EcGlnK sequestration is unaltered in response to an  $\text{NH}_4^+$  shock.

[1] Tremblay, P.L., and Hallenbeck, P.C. "Of blood, brains and bacteria, the Amt/Rh transporter family: emerging role of Amt as a unique microbial sensor" *Mol. Microbiol.* 71: 12-22, 2009; [2] Yakunin, A.F. and Hallenbeck, P.C. "AmtB is necessary for  $\text{NH}_4^+$ -induced nitrogenase switch-off and ADP-ribosylation in *Rhodobacter capsulatus*" *J. Bacteriol.* 184:4081-4088, 2002; [3] Tremblay, P.-L., Drepper, T., Masepohl, B., and Hallenbeck, P.C. "Membrane sequestration of PII proteins and nitrogenase regulation in the photosynthetic bacterium *Rhodobacter capsulatus*" *J. Bacteriol.* 189, 16, 5850-5859, 2007; [4] Tremblay, P.-L., and Hallenbeck, P. C., "Ammonia-induced Formation of an AmtB-GlnK Complex is not sufficient for Nitrogenase Regulation in the photosynthetic bacterium *Rhodobacter capsulatus*", *J. Bacteriology*, 190:1588-1594, 2008

## OC51: Iron and manganese in cyanobacteria: a story of co-regulation

**Nir Keren, Shir Kerem, Chana Kranzler, Eitan Salomon and Hagit Zer**

Department of Plant and Environmental Sciences, the Alexander Silberman Institute of Life Sciences, Edmond J. Safra Campus, Givat Ram, Hebrew University of Jerusalem, Jerusalem, Israel

In many aquatic environments iron concentrations limit cyanobacterial growth. Research on iron uptake mechanisms of cyanobacteria has focused on endogenous siderophore production and internalization. However, as many cyanobacterial species do not produce siderophores (*Synechocystis* sp. PCC 6803 included), alternative Fe acquisition mechanisms must exist. *Synechocystis* 6803 is capable of acquiring iron from exogenous ferrisiderophores (Ferrioxamine-B, FeAerobactin). Unchelated, inorganic Fe is a highly available source of iron. Inhibition of iron uptake by the Fe(II)-specific ligand, ferrozine, indicated that reduction of both inorganic iron and ferrisiderophore complexes occurs prior to transport through the plasma membrane. Analysis of mutant strains indicated that the major iron transporter in the plasma membrane is the FeoB, an Fe(II) channel. The source of reductive power is linked to the respiratory chain. The reduction based uptake strategy is well suited for acquiring iron from multiple complexes in dilute aquatic environments and may play an important role in other cyanobacterial strains.

Mn represents a very different scenario. Mn ions are essential for oxygen evolution but their concentrations in natural environments is rarely limiting. Cultures of *Synechocystis* 6803 could grow, without any observable effect on their physiology, on Mn concentrations as low as 100 nM. Below this threshold a decline in the photochemical activity of photosystem II (PSII) occurred. Surprisingly, the decrease in PSII activity was paralleled by a decrease in the photosystem I (PSI) activity. This decrease did not result from a reduction in total PSI content. BN-PAGE analysis revealed that the cause of this reduction is a Mn limitation dependent dissociation of PSI trimers into monomers. The sensitive range for changes in the organization of the photosynthetic apparatus overlaps with the range of Mn concentrations measured in natural environments. We suggest that the ability to manipulate the organization of PSI allows cyanobacteria to balance electron transport rates between the photosystems.

Finally, we have found that the homeostasis of iron and Mn are connected to each other. Iron limitation induces a reduction in the cellular quotas of both Fe and Mn. The response is specific and unidirectional. Mn limitation does not induce a decrease in Fe quotas. The Fe induced Mn reduction is sufficient to induce changes in the function of the photosynthetic apparatus as described above for Mn limiting conditions. We propose that this coordinated reduction helps reduce oxygen evolution and electron transport rate from PSII to match the reduced function of PSI, under these conditions.

## OC52: Proteins joining the cells in the filaments of *Anabaena* sp. PCC 7120

**Vicente Mariscal, Victoria Merino-Puerto, Antonia Herrero and Enrique Flores**

Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, Avenida Américo Vespucio 49, E-41092 Seville, Spain

Many cyanobacteria grow as filaments of cells or trichomes in which, under combined nitrogen deprivation, two different cell types are found: the vegetative cells that perform oxygenic photosynthesis and the heterocysts that carry out nitrogen fixation [1]. Heterocysts differentiate from vegetative cells in a process that involves a specific program of gene expression. During the differentiation process and in the developed filament, intercellular exchange of nutrients and regulatory compounds takes place making these cyanobacteria to behave as true multicellular organisms. The cyanobacteria bear a Gram-negative type of cell envelope. In heterocyst-forming cyanobacteria, the outer membrane is continuous along the filament without entering the septa between cells [2]. The periplasm, which lies between the cytoplasmic and outer membranes, is therefore continuous along the filament and could represent a communication conduit between cells [3]. Additionally, some structures that have been termed "microplasmodesmata" appear to be present in the intercellular septa [2]. Electron tomography of the model heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120 has recently confirmed the presence of a continuous periplasm and of intercellular septal structures, which have been termed "septosomes" [4]. In this cyanobacterium, we have identified three proteins, SepJ, FraC and FraD, which are located at the intercellular septa along the filament. Mutants of the genes encoding these proteins show a filament fragmentation phenotype indicating that they contribute to keep cells together in the filament [5,6]. These mutants are also impaired in the intercellular transfer of fluorescent tracers suggesting that the encoded proteins are also involved in intercellular molecular exchange [5,6]. Recent work has shown that the activities of SepJ, on one hand, and of FraC/FraD, on the other, can be differentiated using different tracers. These results suggest that two functionally different types of cell-cell joining complexes are present at the intercellular septa in the filaments of this cyanobacterium [7]. Current attempts at isolating a mutant of *Anabaena* sp. PCC 7120 lacking the two intercellular joining structures will be presented. Our current view of the *Anabaena* filament is that of a string of cells that are encapsulated by a continuous outer membrane and share the periplasm. The cells in the filament are additionally joined by some unique proteinaceous structures that are also involved in intercellular communication.

[1] Flores E. & Herrero A. (2010) Nat. Rev. Microbiol. 8:39-50; [2] Flores E. et al. (2006) Trends Microbiol. 14:439-443; [3] Mariscal V. et al. (2007) Mol. Microbiol. 65:1139-1145; [4] Wilk L. et al. (2011) Cell. Microbiol. 13:1744-1754; [5] Merino-Puerto V. et al. (2010) Mol. Microbiol. 75:1159-1170; [6] Mariscal V. et al. (2011) Mol. Microbiol. 79:1077-1088; [7] Merino-Puerto V. et al. (2011) Mol. Microbiol. 82:87-98



### OC53: Mechanisms and evolution of growth on sulfur compounds in phototrophic bacteria

**Niels-Ulrik Frigaard<sup>1</sup>, Carina Holkenbrink<sup>1</sup>, Lea Gregersen<sup>1</sup>, Thomas Grochalski<sup>1</sup>, Nicola Storelli<sup>2</sup>, Mauro Tonolla<sup>2</sup>, Donald A. Bryant<sup>3</sup>, Raymond P. Cox<sup>4</sup>, and Mette Miller<sup>4</sup>**

<sup>1</sup>Department of Biology, University of Copenhagen, Denmark; <sup>2</sup>Plant Biology Department, University of Geneva, Switzerland; <sup>3</sup>Department of Biochemistry and Molecular Biology, The Pennsylvania State University, PA, USA; <sup>4</sup>Department of Biochemistry and Molecular Biology, University of Southern Denmark, Denmark

Photosynthesis requires a suitable reductant for CO<sub>2</sub> fixation. Cyanobacteria are the only bacteria that use H<sub>2</sub>O as reductant for photosynthesis; a much wider variety of photosynthetic bacteria have specialized in using reduced, inorganic sulfur compounds as reductant. This lifestyle allows these bacteria to grow photoautotrophically in anaerobic environments using reduced sulfur compounds such as sulfide and thiosulfate (*photothiotrophy*). The two major groups of these bacteria are the green sulfur bacteria (GSB) and the purple sulfur bacteria (PSB). Oxidation of sulfur compounds for phototrophic growth is also found sporadically among other types of phototrophs including certain cyanobacteria and filamentous anoxygenic phototrophic bacteria.

To gain insight into the oxidative sulfur metabolism of photothiotrophs, we analyzed the recently available genome sequence information from 15 strains of GSB and 12 strains of PSB. Sulfide:quinone oxidoreductase (SQR) oxidizes sulfide and seems to be present in all photothiotrophs and is apparently involved in both photothiotrophy and sulfide detoxification. Dissimilatory sulfite reductase (DSR) seems to be present in all photothiotrophs that oxidize sulfur globules to sulfate, with a few puzzling exceptions among the *Ectothiorhodospiraceae*. Genome comparisons also showed that these and other sulfur oxidation systems appear to have been shuffled among photothiotrophs and chemolithotrophs by horizontal gene transfer resulting in increased substrate range in the recipient organisms and possibly also in increased conservation of chemical energy.

To investigate the function of the many known and putative sulfur oxidation systems revealed by genome sequence analyses, the corresponding genes were inactivated in the GSB *Chlorobaculum tepidum*. The results showed that SQR provides high-affinity sulfide uptake but is only partially responsible for sulfide oxidation in this organism. The results also suggested that DSR is not important for growth under high sulfide concentrations because the sulfide oxidation by SQR (and additional unknown enzymes) is capable of providing sufficient reductants for growth. Because DSR is the only known system in photothiotrophs that allow complete oxidation of sulfide and sulfur to sulfate, DSR may have been acquired by horizontal gene transfer to allow photothiotrophy at limiting sulfide concentrations.

The oxidative sulfur metabolism of GSB under environmental conditions was also investigated by metatranscriptome sequencing in Lake Cadagno, Switzerland, where a clonal population of *Chlorobium clathratiforme* dominates the anaerobic community in the chemocline. Although surprisingly few genes exhibited strong regulation, DSR was downregulated under high sulfide concentrations as suggested by experiments with axenic cultures of *Chlorobaculum tepidum*.

### OC54: The role of Dps (DNA-binding proteins from starved cells) -like proteins in the adaptations to environmental perturbations in cyanobacteria

**Martin Ekman<sup>1</sup>, Anja Nenninger<sup>1</sup>, Paulo Oliveira<sup>1,2</sup>, and Karin Stensjö<sup>1</sup>**

<sup>1</sup>Department of Chemistry-Ångström Laboratory, Uppsala University, Uppsala, Sweden; <sup>2</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal

All organisms need to adapt to conditions, such as photon flux density, temperature and nutrient availability, that alter the redox milieu of their cells to prevent that toxic levels of reactive oxygen species (ROS) are accumulated which will cause oxidative stress inside the cells. The ability to adapt has an enormous impact for fitness of life and is important for production of biomass for food, feed and bio-fuels. In cyanobacteria a number of proteins are involved in protection against ROS including superoxide dismutases (SODs), flavodiiron proteins, peroxiredoxins and Dps. The Dps have in many bacteria been shown to be involved in iron sequestration, DNA binding, and hydrogen peroxide detoxification. However, very little is known concerning the specificity and redundancy of the Dps proteins in cyanobacteria.

By using quantitative shotgun proteomics analyses we have found that several of the individual proteins in the protein-families involved in control of ROS in *Nostoc punctiforme* ATCC 29133 and *Nostoc* sp. PCC 7120 are specific to either of the two cell types, heterocysts and vegetative cells<sup>1,2</sup>, suggesting cellular- and functional specificity. To test these ideas we are using a multidimensional approach with gene-inactivation, promoter reporter gene studies, over-expression of proteins as well as confocal microscopy analyses and more, with focus on the five Dps-like proteins, which are expressed in *N. punctiforme*. We inactivated each of the five Dps proteins, and the ΔDps1-ΔDps5 mutants show differences in their tolerance against ROS. To investigate *in vivo* activity and strength of the five *dps* promoters, Promoter<sub>dps</sub>-GFP reporter gene fusions have been created. Our results show that the promoters have different strengths, are active under different growth conditions and in different cell types. At least three of the P<sub>dps</sub> demonstrate a heterocyst specific activity. One of the five promoters displays an equal strength of activity in both vegetative cells and heterocysts. The transcript regulated by the latter promoter was the only one of the five *dps* transcripts in *N. punctiforme*, which was strongly induced upon hydrogen peroxide treatment. Furthermore, overexpression of this *dps* in *Escherichia coli* demonstrated increased tolerance against added hydrogen peroxide. Together these results indicate a role in hydrogen peroxide control/detoxification for one of the five Dps investigated. Overall, the results provide new insight into the importance and specificity of Dps proteins in adaptation of *N. punctiforme* to environmental stress conditions.

[1] Ow SY, Cardona T, Taton A, Magnuson A, Lindblad P, Stensjö K, Wright PC (2008), J Proteome Res 7:1615-1628; [2] Ow SY, Noirel J, Cardona T, Taton A, Lindblad P, Stensjö K, Wright PC (2009), J. Proteome Res 8 (1): 187-198.



## OC55: Impairment of O-antigen Production Confers Resistance to Grazing in a Model Cyanobacterium-Amoeba Predator-Prey System

Ryan Simkovsky<sup>1</sup>, Emy Daniels<sup>2</sup>, Karen Tang<sup>1</sup>, Stacey Hyunh<sup>1</sup>, Bianca Brahamsha<sup>2</sup>, and Susan Golden<sup>1</sup>

<sup>1</sup>Division of Biological Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA; <sup>2</sup>Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA

The grazing activity of predators on photosynthetic organisms is one of the primary difficulties in growing cyanobacteria and other microalgae in large, open ponds for the production of biofuels. These contaminants destroy valuable biomass and prevent stable, continuous production of biofuel crops. To address this problem, we have isolated an amoeba, HGG1, that grazes upon unicellular and filamentous freshwater cyanobacterial species. We have established a model predator-prey system using this amoeba and *Synechococcus elongatus* PCC 7942. Application of amoebae to a library of mutants of *S. elongatus* led to the identification of a grazer-resistant knockout mutant of the *wzm* ABC O-antigen transporter, SynPCC7942\_1126. We have shown that mutations in three other genes involved in O-antigen synthesis and transport also prevent the expression of O-antigen and confer resistance to HGG1. Complementation of these rough mutants returns O-antigen expression and susceptibility to amoebae. Rough mutants are easily identifiable by appearance, are capable of autoflocculation, and do not display growth defects under standard laboratory growth condition, all of which are desired traits for a biofuel production strain. We have demonstrated that preventing the production of O-antigen is a major pathway for producing grazer resistance.

.....



# POSTER>SESSION I

## PHYLOGENY, TAXONOMY AND DIVERSITY



## POSTERS> I. PHYLOGENY, TAXONOMY AND DIVERSITY

### P1: Polyphasic characterization of *Dolichospermum uruguayanum* spec. nov., a planktonic cyanobacterium dominating the Lower Uruguay River, South America

**Eliška Zapomělová<sup>1,2</sup>, Graciela Ferrari<sup>3</sup>, María del Carmen Pérez<sup>4</sup>**

<sup>1</sup> Biology Centre of AS CR, Institute of Hydrobiology, Na Sádkách 7, CZ-37005 České Budějovice, Czech Republic; <sup>2</sup> University of South Bohemia, Faculty of Science, Branišovská 31, CZ-37005 České Budějovice, Czech Republic; <sup>3</sup> Department of the Environment, Laboratorio Tecnológico del Uruguay (LATU), Avda. Italia 6201, CP 11500 Montevideo, Uruguay; <sup>4</sup> Phytoplankton Consultant, Calle Busot 6, 1° 46007 Valencia, Spain

The Lower Uruguay River is one of the largest rivers in South America, stretching over 1,800 km and with annual discharge 6230 m<sup>3</sup>.s<sup>-1</sup>. It belongs to the La Plata Basin and its lower part flows between two countries, Argentina and Uruguay. One of the main factors affecting the water quality is the construction of more than twenty hydropower dams. Other human activities, such as increasing urbanization and the expansion of agriculture, have recently affected the flow rate and water quality. These effects, together with climate variations favour the proliferation of massive blooms of planktonic cyanobacteria. The blooms usually contain several *Dolichospermum* morphospecies. One of them, preliminarily reported as *D. cf. pseudocompactum*, often forms a dominant together with *Microcystis aeruginosa* (Ferrari et al. 2011). Morphological characteristics of this morphospecies are similar to *D. pseudocompactum* in some points, but do not fully correspond with its description, neither with definitions of any *Dolichospermum* species described so far. A clonal strain was isolated in 2010 and deposited in culture collection of Biology Centre of AS CR, v.v.i., Institute of Hydrobiology in České Budějovice, Czech Republic. Sequence of its 16S rRNA gene was acquired and compared with 16S rRNA gene sequences of other *Dolichospermum* species available from Genbank, incl. *D. pseudocompactum*. Based on results of three phylogenetic analyses (Maximum Likelihood, Maximum Parsimony and Neighbor Joining), the cyanobacterium from Uruguay do not cluster together with *D. pseudocompactum* strains. We proposed an establishment of a new species *D. uruguayanum*, as it displays unique morphological features and do not significantly cluster with another *Dolichospermum* species based on the 16S rRNA gene sequences.

Ferrari, G., Pérez, M. del C., Dabiezies, M., Míguez, D., Saizar, C. (2011): Planktic cyanobacteria in the Lower Uruguay River, South America. *Fottea* 11: 225-234.

### P2: Inferring the phylogeny of *Anabaena azollae* isolated from *Azolla* species with phycocyanin an 16S

**Ana L. Pereira<sup>1</sup>, Isabel Fernandes<sup>1</sup>, Vítor Vasconcelos<sup>1,2</sup>**

<sup>1</sup>CIIIMAR/CIMAR, LEGE, Universidade do Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal; <sup>2</sup>Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

Some species of cyanobacteria establish symbiosis with plants such as pteridophyte (*Azolla*), the gymnosperm (*Macrozamia*), the angiosperm (*Gunnera*), and others but the only permanent symbiosis is *Azolla-Anabaena azollae*. This symbiosis is widely studied due to their potential applications as biofertiliser, animal food or in the phytoremediation of contaminated wastewater. The filamentous and heterocystous endosymbiont is transmitted throughout the entire life cycle of *Azolla* since it inhabits an ovoid cavity on the *Azolla* dorsal lobes and in a cavity in the megasporocarps. The controversial taxonomy of the endosymbiont arises from the three possible names attributed - *Anabaena azollae*, *Nostoc azollae* and *Trichormus azollae* - depending of the gene analysed (16S or phycocyanin). Another problem that even the genome sequence in 2010 did not solved yet is the possible co-evolution between the *Azolla* species and the endosymbiont. Therefore, the present research analyzed the genetic relatedness and the phylogeny of the endosymbiont *A. azollae* of all *Azolla* species using the 16S and phycocyanin genes. The endosymbionts were isolated from the foliar cavities of 47 *Azolla* specimens using the gentle roller and centrifugation. The trees obtained with both genes and using the Neighbour-Joining, Maximum Parsimony and Maximum Likelihood give two clusters: one grouping the endosymbionts that inhabit the *Azolla* species of section *Rhizosperma* (*A. nilotica* and *A. pinnata*) and the other group with the *Azolla* species from section *Azolla* (*A. filiculoides*, *A. caroliniana*, *A. rubra*, *A. microphylla* and *A. mexicana*). In addition, it was possible to assign each *A. azollae* strain to each *Azolla* species especially, *A. nilotica*, *A. pinnata* and *A. filiculoides* point out to a possible co-evolution between the *Azolla* species and their cyanobiont.

### P3: Phylogeny of “*Leptolyngbya* - like” strains isolated from phyllosphere of the Brazilian Atlantic Forest

**Andreote, A.P.D.<sup>1</sup> and Fiore, M.F.<sup>1</sup>**

<sup>1</sup> Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil

The genus *Leptolyngbya* is a polyphyletic assemblage. One group of strains that fits within the current circumscription of *Leptolyngbya* is genetically and phylogenetically distinct from *Leptolyngbya sensu stricto*. Based on molecular, ultrastructural and ecophysiological criteria, the separation of *Leptolyngbya* in a few different genera is expected. The leaves surface (phyllosphere) is a hostile environment, with limited nutrients and water, and intense climatic variations. Nevertheless, this extreme environment can uphold a huge and poorly known microbial diversity. The aim of this study was evaluated “*Leptolyngbya* - like” strains isolated from Atlantic forest phyllosphere using morphological and phylogenetic approaches. Leaf samples were collected from bamboo (*Merostachys neesii*), juçara palm (*Euterpe edulis*), *Garcinia gardneriana* and *Guapira opposita* at Serra do Mar State Park, São Paulo, Brazil. The mono-specific cultures were obtained by immersing of the leaves followed by repeated streaking into BG-11 medium. Cultured cells were maintained under a 10:14 light:dark (L:D) cycle with white fluorescent light (30  $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$ ) at 25°C. Genomic DNA extractions were performed and PCR amplification of partial 16S rRNA gene (around 1400 bp) was conducted using the primer set 27F1/1494Rc. PCR products were cloned and sequenced. Partial 16S rRNA gene sequences were processed to remove low quality bases (quality score <20) and compared with others deposited in GenBank (NCBI). Phylogenetic reconstruction was carried out using the Maximum likelihood method. In the total, it was isolated and characterized 12 “*Leptolyngbya* - like” strains, being two from bamboo, five from juçara palm, two from *G. gardneriana* and three from *G. opposita* phyllospheres. Plant species and morphological diacritic traits did not affect the phylogenetic affiliation of the new strains into the *Leptolyngbya* group. A high number of cultured and uncultured cyanobacteria sequences from mats, biofilms and extreme environments grouped together with the new sequences. Two clades containing exclusively some of the phyllospheres sequences and sequences from Antarctic environment were formed. This profile assembly illustrates the relevance of the environment (extreme environment) to *Leptolyngbya* phylogeny and taxonomy organization. The 16S rRNA sequences varied from 93% to 99% of similarity with their closest relatives deposited in GenBank. Three strains were below to the cut-off criteria of 95% for genera, and grouped together in a separated clade with some uncultured cyanobacteria. This clade will be better investigated by polyphasic approach. Thereby, this study allowed the phylogenetic characterization of “*Leptolyngbya* - like” strains from Atlantic forest phyllosphere and introduced new insights to the taxonomy of pseudanabaenacean group.

### P4: High Levels of Morphological and Cellular Differentiation in the Filamentous Euendolithic Cyanobacterium *Mastigocoleus testarum* Strain BC008

**Brandon S. Guida<sup>1</sup> and Ferran Garcia-Pichel<sup>1</sup>**

<sup>1</sup>The School of Life Sciences at Arizona State University, Tempe, Arizona USA

*Mastigocoleus testarum* strain BC008 is a filamentous, true-branching, heterocystous cyanobacterium which plays a major role in environmental biogenic erosion of limestones and biogenic carbonates. The exact mechanism of mineral dissolution has only recently begun to be studied. The process occurs through active calcium ion pumping mediated by at least one P-type calcium ATPase pump. To gain a greater understanding of the mechanism behind calcium carbonate boring we utilized various microscopy techniques that have uncovered a surprising level of intrafilamentous cellular heterogeneity, which we think may be functional. Multicellular filament regions seem to be specialized in concentrating calcium and contain little photosynthetic pigmentation. Conversely, regions that contain typical pigmentation autofluorescent profiles, have no detectable calcium signal. We have also observed further cellular diversification regarding phycobiliprotein contents and composition. In addition, we have observed concentrated intracellular accumulations of autofluorescent pigments in specific filament regions and the development of long, thin trichome extensions containing no light harvesting pigmentation. This cellular diversity can be seen within a very small sample of boring as well as in non boring biomass. This level of differentiation within a single species of filamentous cyanobacteria is unprecedented.



## P5: New genus of Phormidiaceae separated from the traditional heterogenus Phormidium

**Camila Francieli da Silva Malone<sup>1,2</sup>, Marli Fátima Fiore<sup>3</sup>, Janaína Rigonato<sup>3</sup>, Zenilda Laurita Bouzon<sup>4</sup> and Célia Leite Sant'Anna<sup>1</sup>**

<sup>1</sup>IBt – Nucleus of Phycology, Institute of Botany, 04301-902, São Paulo, Brazil; <sup>2</sup>PhD Student, Institute of Botany, Brazil, Grant Support of FAPESP (Process 2010/51666-0) cfsmalone@yahoo.com.br; <sup>3</sup>Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, Brazil; <sup>4</sup>Department of Cell Biology, Embryology and Genetics, Federal University of Santa Catarina, Florianópolis, Brazil

The modern taxonomic revision of the traditional cyanobacterial genus *Phormidium* is based on molecular sequencing combined with the definition of distinct morphologic features. Lately, some genera clearly distinct by morphological markers and molecular sequencing (genetic similarity lower than 95%) were already defined and separated from this polyphyletic generic unit (*Phormidesmis*, *Wilmottia*, *Oxynema*). According to 16S rRNA gene sequencing, we are studying a group of morphologically similar species from distinct Brazilian aquatic environments, which represents an isolated cluster with strains identified as *Phormidium tergestinum* (CCALA155) and *Phormidium autumnale* (UTEX1580). Morphologic investigations of the Brazilian strains CCIBt3279 and CCIBt3523 (freshwater aquarium) and CCIBt3277 (alkaline lake of the Pantanal) indicated as diacritical features: cylindrical and straight trichomes, sometimes slightly constrict, hyaline sheaths, firm, attached to a trichome or wide, apical cells strongly or slightly capitated, sometimes bent at the end. All these morphological features were also observed in the strains CCALA 155 and UTEX 1580, supporting their inclusion in a well-defined branch together with the Brazilian strains (100% bootstrap value). Based on the 16S rRNA gene, all the strains analyzed showed high phylogenetic similarity among them (100%). However, the alkaline strain from Pantanal (CCIBt3277) presents trichomes bent at the end and the apical cells more evidently capitated than the aquarium strains (CCIBt3279, CCIBt3523, UTEX1580) and the strain CCALA155 that was growing on concrete surface. Probably, the small morphological differences among the strains should be related to their different habitats. Except for the strain CCALA155 (concrete substrate), all the others may present typical aerotopes. Based on the analysis of the strain CCIBt3277 life cycle, it was possible to define two distinct phases: the initial inoculum composed of trichomes with aerotopes and without mucilaginous sheath showed that after six days there is a initial loss of aerotopes and conspicuous hyaline sheaths are formed. In the last phase the hormogonium are formed and released from the sheath and aerotopes begin to appear again. This cycle shows that in nature, trichomes can have a planktonic phase with aerotopes and a benthic phase without aerotopes. The same strain was studied under a transmission electronic microscope and showed the presence of aerotopes and the radial position of the thylakoids, typical of the Phormidiaceae family. The maximum likelihood tree of 16S rRNA gene sequences shows that the new clade displays less than 90% sequence similarity with the recently described *Phormidesmis*, *Wilmottia*, *Oxynema* and with the genus *Planktothrix* in which the aerotopes are the main diacritic feature. Base on our results, the studied strains represent a new genus phylogenetically and morphologically distinct from *Phormidium* and from other close genera.

## P6: Evaluation of phylogenetic markers suitable for *Planktothrix* spp. discrimination

**Carina Menezes<sup>1</sup>, Catarina Churro<sup>1,2</sup>, Paulo Pereira<sup>1</sup>, Vítor Vasconcelos<sup>2,3</sup> and Elisabete Valério<sup>1,3</sup>**

<sup>1</sup>Laboratório de Biologia e Ecotoxicologia, Departamento de Saúde Ambiental, Instituto Nacional de Saúde Dr. Ricardo Jorge, Avenida Padre Cruz, 1649-016 Lisboa, Portugal; <sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 4069-007 Porto; <sup>3</sup>Laboratório de Ecotoxicologia, Genómica e Evolução, Centro Interdisciplinar de Investigação Marinha e Ambiental, CIIMAR/CIMAR, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal

In Portugal, potentially toxic cyanobacteria from *Planktothrix* genus have become frequently observed in freshwater reservoirs. Identification of *Planktothrix* species through optical microscopy is complicated due to limited morphological differences among them. The aim of this work was to determine the most suitable phylogenetic markers that could be used for the molecular identification of *Planktothrix* species. In order to do so, several genes of interest were selected: *rpoB*, *rpoC1*, *cpcA*, *cpcB*, *rbxX*, 16S rRNA genes and 16S rRNA-tRNAIle-tRNAAla-23S rRNA internal transcribed spacer (ITS), and their sequences retrieved from public databases. Phylogenetic analysis showed that 16S rRNA, *cpcA*, *rbxX* genes and ITS region trees do not allow a clear discrimination of *Planktothrix* species, however *cpcB* and *rpoB* seemed putative suitable phylogenetic markers for *Planktothrix* species identification. The applicability of these markers was then evaluated in 20 *Planktothrix* isolates, isolated over the years from several Portuguese freshwater reservoirs and maintained in the Estela Sousa e Silva Algae Culture Collection (ESSACC). The selected genes, *cpcB* and *rpoB*, were amplified by PCR and sequenced and the resulting trees compared with the phylogenetic clustering obtained with our previously characterized *rpoC1* phylogenies. The phylogenetic analyses, based on the three gene regions, revealed that *Planktothrix* isolates analyzed in this study could be phylogenetically resolved into their corresponding species. This work contributes for the discussion of the appropriate genes that can be used in phylogenetic identification of *Planktothrix* species.

## P7: An early-branching microbialite cyanobacterium forms intracellular carbonates

**Estelle Couradeau<sup>1,2,3</sup>, Karim Benzerara<sup>1</sup>, Emmanuelle Gerard<sup>3</sup>, David Moreira<sup>2</sup>, Sylvain Bernard<sup>4</sup>, Gordon E. Brown Jr.<sup>5</sup> and Purificacion Lopez-Garcia<sup>2</sup>**

<sup>1</sup> IMPMC, CNRS UMR 7590, Université Pierre et Marie Curie, Paris, France; <sup>2</sup> ESE, CNRS UMR 8079, Université Paris-Sud, Orsay, France; <sup>3</sup> IPGP, CNRS UMR 7154, Université Paris Diderot, Paris, France; <sup>4</sup> LMCM - MNHN, CNRS UMR 7202, Paris, France; <sup>5</sup> Department of Geological and Environmental Sciences, Stanford University, Stanford, USA

Cyanobacteria have played a major role in the global carbon cycle for several billion years. They contribute to the 'biological pump' through the photosynthetic conversion of CO<sub>2</sub> to organic matter and its subsequent sinking. Moreover, they can induce calcium carbonate precipitation by photosynthetic consumption of CO<sub>2</sub> and are thus commonly considered as central actors in the formation of sedimentary deposits such as stromatolites or carbonate muds during ephemeral large-scale carbonate precipitation episodes called whittings in marine or lake water columns. It is generally assumed that cyanobacterial calcification is an extracellular process. However, the diversity of mechanisms involved in carbonate precipitation by diverse cyanobacteria is not fully explored yet. Here, we report the discovery of a cyanobacterium found in microbialites from Lake Alchichica (Mexico) [1,2] that forms intracellular carbonate phase inclusions, revealing an unexplored pathway for calcification. Electron diffraction shows that these phases are amorphous. X-ray absorption near edge structure (XANES) nanospectroscopy reveals local ordering consistent with the structure of benstonite, a Mg-Ca-Sr-Ba carbonate. Phylogenetic analyses place this cyanobacterium within the deeply divergent order Gloeobacterales. Accordingly, we tentatively name it *Candidatus Gloeomargarita lithophora*. We suggest that this new process of intracellular carbonate precipitation is a lateral consequence of the photosynthetic activity related to a limited ability to export outside the cell the excess amount of OH<sup>-</sup> derived from conversion of HCO<sub>3</sub><sup>-</sup> into CO<sub>2</sub>.

[1] Couradeau et al., (2011) PLoS ONE 6(12): e28767; [2] Couradeau et al., (2012) Science Vol. 336 no. 6080 pp. 459-462

## P8: Phenotypic and molecular studies of a new cyanobacterial genus from the Atlantic Rainforest, Southeast, Brazil.

**Guilherme Scotta Hentschke<sup>1,2</sup>, Célia L. Sant'Anna<sup>2</sup>, Marli Fátima Fiore<sup>3</sup>, and Janaina Rigonato<sup>4</sup>**

<sup>1</sup>PhD Student, Institute of Botany, Brazil, grant support of National Research Council (CNPq); <sup>2</sup>Institute of Botany, Nucleus of Phycology, São Paulo, SP, Brazil; <sup>3</sup>Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil; <sup>4</sup>Universidade Estadual Paulista, Institute of Biosciences, São José do Rio Preto, SP, Brazil

The Atlantic rainforest is one of the most important hotspots of biodiversity in the world, but the diversity of microorganisms, especially those from terrestrial habitats, remains almost unknown. Because of that, the number of specific publications resulting in descriptions of new cyanobacterial taxa from this biome has increased a lot in the last ten years. Thus, our aim is to describe a new Nostoclean genus, belonging to the family Microchaetaceae, based on phenotypic and molecular data. Samples were collected in the State Park of Ilha do Cardoso (25°04'12"S and 47°55'27"W), sub-tropical area of the Atlantic Rainforest. The studied populations grew in biofilms on rocks and wood and were collected by scraping the substrate with a spatula. Part of the samples were kept dry in paper bags, and a small part was preserved in formaldehyde 4% and held in the Herbarium of the Institute of Botany (SP), Brazil. The strains were isolated in monospecific cultures and kept in the Culture Collection of the Botanical Institute (CCIBt), São Paulo, Brazil under numbers CCIBt 3318 and 3536. The strains are being maintained in BG-11 medium without nitrogen, under controlled conditions: temperature 23 °C, irradiance 40-50 photons m<sup>-2</sup>.s<sup>-1</sup> and photoperiod 14-10 h light/dark. The morphology of the populations from nature and culture were studied under optical microscopy (n=20). The molecular evaluation was done by the 16S rDNA gene sequence that was analyzed by the Maximum Likelihood method, with 1000 bootstrap replicates performed. The molecular analysis of similarity (p-distance) with morphologically related genera was also evaluated. The features of the new genus are constituted by creeping entangled filaments (11-14,5µm wide) in the basal part, becoming erect and parallel toward the ends, forming a short mat. Tolypotrichoid branches are often present near the apex. The trichomes are heteropolar, mostly cylindrical and not constricted. Sheaths are yellowish and lamellated. This new genus is morphologically similar to *Streptostemon*, but differs mainly by the organization of the filaments, not forming well-defined fascicles like *Streptostemon*, and by the presence of isolated false branches near the apex, not found in *Streptostemon*. It differs from *Tolypothrix* by the parallel arrangement of filaments. The phylogenetic tree shows that the strains of the new genus are in a well supported cluster (bootstrap value 99%), that is nested in a major clade formed by Microchaetaceae, Nostocaceae and Rivulariaceae strains and characterized by high bootstrap values (93%). The similarity values with *Tolypothrix* strains are <96% and there are no molecular data about *Streptostemon*. Thus, based on phenotypic and molecular data we are proposing a new terrestrial genus of Cyanobacteria from Brazilian subtropical forest.

## P9: Worldwide comparison of the molecular and morphological diversity within the family Scytonemataceae (Nostocales, Cyanobacteria).

Markéta Bohunická<sup>1,2</sup>, Jan Mareš<sup>1,2</sup>, and Jiří Komárek<sup>1,2</sup>

<sup>1</sup>Department of Botany, Faculty of Science, University of South Bohemia, Branišovská 31, CZ-37005, České Budějovice, Czech Republic; <sup>2</sup>Centre for Phycology, Institute of Botany AS CR, Dukelská 135, CZ-37982, Třeboň, Czech Republic

Members of the cyanobacterial order Nostocales, also called heterocytous cyanobacteria, represent a group of particular interest to both basic and applied research as they possess unique ability to fix atmospheric nitrogen. Family Scytonemataceae within the order Nostocales is traditionally characterized by isopolar filaments with meristematic zones and false branching, and includes genera *Brasilonema*, *Kyrtuthrix*, *Petalonema*, *Scytonema* and *Scytonematopsis* [1, 2]. As a whole, the group of Scytonemataceae is definitely understudied and our knowledge is rather fragmentary. In phylogenetic analyses, the few sequenced members of this family fall to various basal positions in Nostocales, usually with low branch support [3, 4].

To provide more thorough insight into the diversity, distribution and phylogenetic relationships of the taxa within the family Scytonemataceae we gathered unicyanobacterial strains, which were isolated from the samples collected mainly in terrestrial biotopes of all climatic zones and continents excluding Antarctica. Most of the strains represented relatively common cosmopolitan genus *Scytonema* and pantropical *Brasilonema* (ca 100 strains), whereas only few strains of rare genera *Petalonema* and *Scytonematopsis* and no strain of mediterranean *Kyrtuthrix* were acquired. Morphology of the strains was studied under the light microscope and molecular phylogeny of the group and its position within the order Nostocales was assembled based on 16S rDNA. The morphologically well defined genus *Brasilonema* was found to be monophyletic, whereas within the traditional genus *Scytonema*, at least four separated clusters can be recognized and specific morphologic features can be assigned to each of the clusters.

[1]Geitler L. (1932): Cyanophyceae. – In: Rabenhorst's Kryptogamenflora von Deutschland, Österreich und der Schweiz 14: 1-1196, Akad. Verlagsges., Leipzig; [2] Komárek J. & Hauer T. (2011): Cyanodb.cz – On-line database of cyanobacterial genera. – Word-wide electronic publication, Univ. of south Bohemia & Inst. of Botany AS CR, <http://www.cyanodb.cz>; [3]Rajaniemi P., Hrouzek P., Kaštovská K., Williams R., Rantala A., Hoffmann L., Komárek J. & Sivonen K. (2005): Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). – *Int. J. of Syst. Evol. Microbiol.* 55: 11-26; [4]Fiore M.F., Sant'Anna C.L., Azevedo M.T.P., Komárek J., Kaštovský J., Sulek J. & Lorenzi A.S. (2007): The cyanobacterial genus *Brasilonema* – molecular and phenotype evaluation. – *J. Phycol.* 43(4): 789-798

## P10: The photosystem II reaction center D1 proteins in *Arthrospira*

Paul J. Janssen

SCK•CEN – Belgian Nuclear Research Center, B-2400 Mol, Belgium

Photosystem II (PSII) in cyanobacteria is located at the thylakoid membrane and consists of around 20 different subunits depending on the species. A crucial component of this complex is the transmembrane protein D1. This protein holds the Tyr-161 residue (TyrZ) which is directly involved in the reduction of chlorophyll and the liberation of electrons from water in a four-step process producing molecular oxygen. Whereas the other PSII subunits remain relatively undamaged the D1 protein is particularly prone to photo-oxidative damage and needs to be replaced constantly by newly synthesized copies. In addition to this repair cycle, the D1 protein in cyanobacteria is encoded by a multigene *psbA* family encoding D1 isoforms that are expressed in response to environmental cues such as light intensity, temperature, oxygen levels, or UV-exposure. Furthermore, PSII assembly, photosynthetic activity, hydrogen carbonate binding, herbicide tolerance, and tolerance to UV and ionizing radiation have been attributed to specific aminoacid residues in D1.

*Arthrospira* are photoautotrophic filamentous cyanobacteria that typically reside in alkaline lakes rich in carbonates and high in pH and salinity. They have a high nutritional value and are used worldwide as feed for fish, poultry, and farm animals. There is also a growing interest in *Arthrospira* for their possible applications in biofuel technology and CO<sub>2</sub> mitigation. Since photosynthetic yield drives cyanobacterial growth, it is important to study the aminoacidic information of D1 isoforms in *Arthrospira*.

We compared the D1 protein sequences of over 70 cyanobacteria by multiple sequence alignment and performed phylogenetic analyses. All D1 proteins encoded by the four *Arthrospira* draft genomes have a glutamate at position 130 (E130), which is indicative for D1 isoforms expressed under high light conditions. Each of the four *Arthrospira* species possess two isoforms we tentatively call nD1 and aD1 to make a distinction with existing nomenclature, i.e. mD1, D1:1, D1:2, D1', and rD1, since this classification of D1 isoforms cannot be purely sequence based but requires also expression data. The number of *psbA* genes per species for nD1 varied from one to three while each species only had one *psbA* gene encoding the aD1 protein. The nD1 and aD1 sequences grouped tightly together at 99.7% sequence identity or higher in two separate clusters. On average, the two *Arthrospira* isoforms nD1 and aD1 differed in 44 residues from each other. The aD1 sequences had >90% identity to D1' isoforms of *Synechocystis* strain PCC 6803, *Synechococcus* strains RS9917 and CB0101, and *Cyanobium* strain PCC 7001 and displayed features typical for D1' isoforms, including Leu-158, Ser-162, Leu-186, and Ala-286 residues. Unique to *Arthrospira* aD1 proteins as compared to any other D1 protein in the dataset are the pair Arg-235/Arg-238 and a C-terminus consisting of Lys-249/Asn-352/Asn-357. These and other features of aD1 are discussed.

## P11: The Phylogeny of Cyanobacteria Associated with Marine Sponges from Portuguese Coast

Sofia Costa<sup>1</sup>, Ana Regueiras<sup>1,2</sup>, Anoop Alex<sup>1</sup>, Agostinho Antunes<sup>1</sup> and Vítor Vasconcelos<sup>1,2</sup>

<sup>1</sup>CIMAR/CIMAR – Laboratory of Ecotoxicology, Genomics and Evolution – Centre of Environment and Marine Research, University of Porto, Porto, Portugal; <sup>2</sup>Department of Biology, Sciences Faculty, University of Porto, Porto, Portugal

Marine sponges represent a significant component of benthic communities throughout the world. A wide range of organisms are associated with marine sponges, with the most commonly identified being cyanobacteria. Sponges produce a range of chemical compounds and many interactions between sponges and other organisms are mediated through chemical production. A number of cyanobacteria associated with sponges are known, belonging to the genera *Synechocystis*, *Aphanocapsa*, *Oscillatoria*, *Anabaena* and *Synechococcus*. Cyanobacteria can contribute to the host sponge by producing secondary metabolites which may function as defensive compounds. Cyanobacteria benefit sponges through fixation of atmospheric nitrogen. For this study, fragments from the sponge body were used for culturing cyanobacteria. These sections were placed in two different media (Z8 and MN). Pure cyanobacteria cultures were achieved after several subcultures. Partial 16S rRNA gene sequences were obtained for all the isolates. A phylogenetic analysis with 16S rRNA gene fragment was performed to assess the relative positioning of the isolated cyanobacteria from sponges of the Atlantic coast of Portugal, with free-living and sponge-associated cyanobacteria. The sequences used in this analysis were chosen to include (1) representatives of cyanobacterium diversity, (2) sponge-associated cyanobacterium sequences overlapping with the new 16S rRNA sequences, and (3) representatives of the cyanobacteria sponge symbionts. Phylogenetic reconstructions of the 16S rRNA nucleotide data set were performed using Maximum Likelihood (ML) approach implemented in PhyML. The results show sponge-associated cyanobacteria as a polyphyletic group, representative of the genera *Synechococcus*, *Nodosolina*, *Pseudanabaena*, *Phormidium*.

## P12: Novel isolates of limnic aerobic anoxygenic phototrophic bacteria suggest a complex evolution of this functional group

Yonghui Zeng<sup>1</sup>, and Michal Koblížek<sup>1, 2</sup>

<sup>1</sup>Institute of Microbiology CAS, Opatovický mlýn, 379 81 Třeboň, Czech Republic; E-mail: marinezeng@gmail.com; <sup>2</sup>University of South Bohemia, Faculty of Science, Branišovská 31, 37005 České Budějovice, Czech Republic; E-mail: koblizek@alga.cz

Aerobic anoxygenic phototrophs (AAPs) are prokaryotic organisms containing reaction centers composed of bacteriochlorophyll *a* (BChl *a*)<sup>[1]</sup>. AAPs represent an important part of microbial communities inhabiting the euphotic zone of world oceans<sup>[2]</sup> and lakes<sup>[3]</sup>. In contrast to the extensive studies of marine AAPs, only little is known about their presence and diversity in limnic habitats. A recent study of the diversity of AAP in a German lake suggested that freshwater species are formed mostly by Alpha- and Betaproteobacteria<sup>[4]</sup>. So far only a limited number of limnic AAPs exist in pure cultures, which constrains our knowledge on their metabolism, physiology and their role in biogeochemical fluxes. For this reason we attempted to isolate representative AAP species from various limnic environments. We developed a rapid isolation protocol which employs fluorescence screening of AAP colonies directly on agar plates using an infrared CCD camera. We successfully isolated over 200 AAP strains from different types of freshwater lakes located in Austria, Czech Republic and China (a fishpond, a low-elevation volcanic lake, a highly contaminated lake, and a clear Tibetan plateau lake). Most of the strains were affiliated with Alphaproteobacteria belonging to genera *Afiplia*, *Agrobacterium*, *Bosea*, *Brevundimonas*, *Methylobacterium*, *Novosphingobium*, *Phyllobacterium*, *Porphyrobacter*, *Rhizobium*, *Rhodocista*, *Rhodopseudomonas*, *Sandarakinorhabdus*, and *Sphingomonas*. Nine isolates were Betaproteobacteria belonging to genera *Caenimonas*, *Ideonella*, *Leptothrix*, and *Methylibium*. Interestingly, we also isolated one Gammaproteobacterial AAP strain from the Huguangyan Maar Lake, South China, which has not been reported from a freshwater lake before. Tentatively this isolate might establish a new genus within *Gemmatimonadetes*, as its 16S rRNA sequence shows only 80% similarity to that of the type strain *Gemmatimonas aurantiaca* T-27. An analysis of *pufL*, *pufM*, and *BchY* genes sequence suggests a complex phylogenetic history of phototrophic genes in AAP species. Currently ongoing full genome sequencing of representative strains shall provide further insights into the evolution of AAPs and their photosynthetic properties.

[1] Yurkov, V.V., and Csotonyi, J.T. (2009) New light on aerobic anoxygenic phototrophs. In *The Purple Phototrophic Bacteria*, Advances in Photosynthesis and Respiration, Vol. 28. Hunter, C.N., Daldal, F., Thurnauer, M.C., and Beatty, J.T. (eds). Dordrecht, The Netherlands: Springer Verlag, pp. 31–55; [2] Kolber, Z.S., Van Dover, C.L., Niederman, R.A., and Falkowski, P.G. (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature* 407: 177–179; [3] Mašín, M., Nedoma, J., Pechar, L., and Koblížek, M. (2008) Distribution of aerobic anoxygenic phototrophs in temperate freshwater systems. *Environ Microbiol* 10: 1988–1996; [4] Salka I, Čuperová Z, Mašín M, Koblížek M, and Grossart H-P (2011) Rhodoferrax-related *pufM* gene cluster dominates the aerobic anoxygenic phototrophic communities in German freshwater lakes. *Environ Microbiol* 13: 2865–2875

## P13: Species specific analyses of environmental communities of phototrophic bacteria using functional gene sequences

Johannes F. Imhoff

Helmholtz Centre for Ocean research Kiel, Düsternbrooker Weg 20, D-24105 Kiel, Germany

From the establishment of proper cultivation conditions of phototrophic sulfur bacteria 50 years ago up to today significant improvements have been made to systematically treat the phototrophic green and purple sulfur bacteria and identify the species in environmental communities. Important milestones for these improvements were the description of a large number of pure cultures representing a proper fraction of the diversity in nature, their correct taxonomic treatment and the clear definition of the taxa. Further important steps were the establishment of a taxonomy congruent with the phylogeny of 16S rRNA gene sequences and the demonstration of congruence between phylogenies based on 16S rRNA genes and other, functional genes. The formation of large databases of *fmoA* genes of green sulfur bacteria and of *pufLM* genes of purple sulfur bacteria and their obvious phylogenetic congruence with the 16S rRNA gene enabled detailed studies of environmental communities of these bacteria and the recognition of species and genera in natural habitats. Comprehensive studies of selected habitats yielded promising results and demonstrated the potential of this approach for the systematic characterization of environmental communities [1].

[1] Imhoff, J.F.: Functional gene studies of pure cultures are the basis of systematic studies of environmental communities of phototrophic bacteria and their species specific analyses. *BISMIS Bulletin* 2, 107–115 (2011).



## P14: Heterotrophic bacteria in a consortium with the bichlorophyllous cyanobacterium *Prochlorothrix hollandica*

S.G. Averina, Y.V. Chernomorskaya, N.V. Velichko, N. M. Lee\*, and A.V. Pinevich

Department of Microbiology, St.-Petersburg State University, St.-Petersburg, Russia; \*Department of Microbiology, Technical University of München, München, Germany

Prokaryotic consortia (relatively stable cocultures/natural associations or epibiotic aggregates of partners belonging to different genera) are of high ecological and practical significance. However, consortia with the participation of cyanobacteria are poorly investigated (Stevenson, Waterbury, 2006), especially those of bichlorophyllous cyanobacteria (= containing chlorophylls *a/b*), in particular, *Prochlorothrix* spp. (Pinevich et al., 2012).

*P. hollandica* PCC 9006, with only one exception (Schyns et al., 1997), is generally maintained in mixed cultures with heterotrophic bacteria (Pinevich et al., 2012). All attempts to remove these impurities result in a retarded growth and, finally, in the decline of cyanobacterial viability indicating a consortium. Noteworthy, «helper» heterotrophic bacteria associated with another chlorophyll *a/b*-containing cyanobacterium, *Prochlorococcus marinus*, are shown to protect it from oxidative stress and supply it with essential ligands and metallophores (Post, 2006; Moore et al., 2007; Morris et al., 2008).

Our results of fluorescence in situ hybridization (FISH) with universal probes EUBI-III,  $\alpha$ 986,  $\beta$ 42a, and CYF319 demonstrate the heterotrophic partners in a consortium with *P. hollandica* PCC 9006 to be alpha-, beta-, and gamma-proteobacteria. Elective cultures of these bacteria were obtained after streaking serial dilutions (10<sup>-1</sup> to 10<sup>-7</sup>) of the consortium onto BG-11 medium supplemented with 10% (v/v) autoclaved culture of *P. hollandica*. After incubation of agar plates for 7–10 days at 20–22°C, 17 strains originating from three dominating types of colonies (yellow/slimy/3–5 mm; white/opaque/2–3 mm; colorless/transparent/ $\leq$ 1 mm) were obtained. All of them were gram-negative rods with a +/- reaction in the oxidation/fermentation (OF) test. Bacteria from «yellow» colonies were catalase- and oxydase-positive, while those from «white» and «colorless» showed positive reaction for catalase only.

To prove the affiliation of heterotrophic partners, the PCR analysis of 16S rRNA gene using bacterial universal 27f and 1492r primers was performed. The database alignment of cloned and sequenced amplicons demonstrated the strains from «yellow» colonies to belong to the genus *Pseudomonas* (gamma-proteobacteria), the strains from «white» morphotype – to the group *Rhizobium/Agrobacterium* (alpha-proteobacteria), and the strains from «colorless» morphotype – to the group *Pelomonas/Roseateles/Paucibacter* (beta-proteobacteria). These data are in agreement with FISH-experiments. To check the common nature of strains, purified 1450bp-PCR fragments were treated with restrictases RsaI, AluI, and HhaI. Restriction profiles within the individual morphotypes proved mutually similar.

To summarize, we firstly identified heterotrophic partners in the consortium of *P. hollandica*. Detailed study of mechanisms which underlie corresponding physiological interactions is in progress.

Literature cited: 1) Morris J.J., Kirkegaard R., Szul M.J. et al. // Appl. Environ. Microbiol. 2008. Vol. 74. P. 4530–4534; 2) Moore L.R., Coe A., Zinser E.R. et al. // Limnol. Oceanogr. 2007. Vol. 5. P. 353–362; 3) Pinevich A., Velichko N., Ivanikova N. // Frontiers in Microbiol. Vol. 3. doi: 10.3389/fmicr.2012.00173; 4) Post A.F. // In: Prokaryotes. Vol. 4 / Eds. M. Dworkin et al. NY. Springer-Verlag. 2006. P. 1099–1110; 5) Schyns G., Rippka R., Namane A. et al. // Res. Microbiol. 1997. Vol. 148. P. 345–354; 6) Stevenson S.E., Waterbury J.B. // Biol. Bull. 2006. Vol. 210. P. 73–77.

## P15: Characterization of planktonic non sulfur purple bacteria, isolated from the Campeche Canyon (Gulf of México)

<sup>1</sup>María Teresa Núñez-Cardona, <sup>1,2</sup>Verónica Pacheco González, <sup>2</sup>Jaime García Mena

<sup>1</sup>UAM-X- Universidad Autónoma Metropolitana-Xochimilco; <sup>2</sup>CINVESTAV-Z-Centro de investigaciones y de estudios avanzados-Zacatenco

Marine photosynthetic bacteria thrive in all kinds of shorelines and geographical regions and also in stratified water bodies such as fjords, deeper bays and lagoons[1]. Although, they have been detected using molecular techniques, this has not been enough to know the role of these microorganisms at marine environments. With the aim to isolate purple non sulfur photosynthetic bacteria (NSPB), enrichments were done with water samples collected at 100 m depth at the Campeche Canyon (Gulf of México). Using a selective culture medium for NSPB it was possible to isolate NSPB. After repeated agar shake techniques we got pure cultures and they were identified using some of the standard recommendations for description of new photosynthetic anoxygenic bacteria species[2] like *in vivo* pigment composition analysis (extracted pigments were analyzed too), the use of different carbon sources, and the analysis of 16S rDNA. Four pure cultures were obtained, they are rod-shape and, their photosynthetic pigments show *in vivo* absorbance characteristics of bacteriochlorophyll *a* (377-379, 590, 806, 865-867 nm) and carotenoids of the normal spirilloxanthin series (493-495; 522 nm); Bchl *a* was also detected in methanol extracts at 364 and 771 nm. Carbon and energy sources for these cultures could be acetate, propionate, mannose, glycerol, yeast extract, maltose, pyruvate and succinate, and they do not use or their growth is limited in mannitol, methionine, glycine, thiosulfate, cysteine, glucose, fructose, lactose, ethanol and methanol. Although the strains isolated showed differences in the use of organic carbon and energy sources, 16S rDNA analysis revealed that they are members of *Rhodospseudomonas* genus. DNA extraction of the strains have been achieved and amplified by PCR technique, this result was analyzed by electrophoresis in a gel of agarose (1.0%); a BLAST and by phylogenetic analyses based on 16S rDNA gene sequence which showed that the four isolates were related with *Rhodospseudomonas* spp. Members of genus *Rhodospseudomonas* have been isolated from marine sponges[3] the exit of this genus in nature is due, in part, because its metabolic capabilities and that it is able to grow in aerobic (without pigment productions) and anaerobic conditions.

[1] Imhoff JF. 2001. True marine anaerobic phototrophic bacteria. Archives of Microbiology, 176 (4):243-254; [2] Imhoff, JF, Caumette P. (2004). Recommended standards for the description of new species of anoxygenic phototrophic bacteria, International Journal of Systematic and Evolutionary Microbiology, 54 (4):1415-1421; [3] Imhoff JF, Trüper HG. 1976. Marine sponges as habitats of anaerobic phototrophic bacteria. Marine Ecology, 3 (1):1-9

**P16: Genetic diversity of the invasive cyanobacterium *Cylindrospermopsis raciborskii* from a Tunisian reservoir****Afef Fathalli<sup>1</sup>, Amel Ben Rejeb Jenhani<sup>1</sup>, Cristiana Moreira<sup>2</sup>, Agostinho Antunes<sup>2</sup>, Martin Welker<sup>3</sup>, Mohamed S. Romdhane<sup>1</sup>, Vitor Vasconcelos<sup>2,4</sup>**<sup>1</sup>Unité de Recherche Ecosystèmes et Ressources Aquatiques, Institut National Agronomique de Tunisie, Tunisia; <sup>2</sup>Centro de Investigação Marinha e Ambiental- CIIMAR/CIMAR, Universidade do Porto, Porto, Portugal; <sup>3</sup>Technische Universität Berlin, Berlin, Germany; <sup>4</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Portugal

*Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju is a freshwater cyanobacterium of tropical origin also found in temperate regions [1]. Due to its known ability to produce potent toxins such as the cytotoxin cylindrospermopsin [2] and the neurotoxic paralytic shellfish poisons [3], this species is of major concern from a water quality perspective. In Tunisia, this species has been reported for the first time at Bir M'cherga reservoir, in October 2004. Five *Cylindrospermopsis raciborskii* strains, isolated from this reservoir during the period 2005 to 2008, have been molecularly characterized. These strains, were genetically very different according to the PCR analyses targeting genes coding for the cylindrospermopsins, saxitoxins and microcystins. In fact, we noted that one isolated *Cylindrospermopsis raciborskii* strain presented *mcyA* and *mcyE*, from the six characteristic segments of the microcystin synthetase *mcy* cluster (*mcyA*, *mcyB*, *mcyC*, *mcyD*, *mcyE*, and *mcyG*). This strain was found not microcystin-producing by MALDI-TOF analysis. None of all other studied strains were microcystin-producing, neither saxitoxin-producing nor cylindrospermopsin-producing although there was a registered presence of PS and PKS genes in some strains. The compilation of the phylogenetic data based on the 16 S rRNA and *rpoC1* genes from this work and from the GenBank database revealed the genetic variability of the Tunisian isolates. In fact, according to the 16S rRNA sequences, the strain presenting *mcyA* and *mcyE* seems divergent from other *Cylindrospermopsis raciborskii* strains worldwide. The *rpoC1* gene, more discriminatory at the species level than the 16 S rRNA, revealed that all Tunisian isolates clustered together but with high genetic variability compared to the other African strains. This different clustering of the African strains demonstrates that the population structure in this continent is somewhat heterogeneous, supporting the uniqueness of the Tunisian isolates relatively to *Cylindrospermopsis raciborskii* strains from other geographical locations.

[1] Padisak (1997). *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding highly adaptive blue-green algal species, worldwide distribution and review of its ecology. Arch Hydrobiol 107 (Suppl) :563-593; [2] Hawkins P.R., Runnegar M. T. C., Jackson A. R. B., Falconer I. R. (1985). Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green algae) *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju isolated from a domestic Water supply reservoir. Appl. Environ. Microbiol. 50:1292-1295; [3] Lagos N., Onodera H., Zagatto P.A., Andrinolo D., Azevedo S.M.F.O, Oshima Y (1999). The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. Toxicon 37: 1359-1373

.....

# POSTER>SESSION II

## ENVIRONMENT AND ECOLOGY





## POSTERS> II. ENVIRONMENT AND ECOLOGY

### P17: *Cylindrospermopsis*: features explaining its success in Brazilian aquatic system

**Andreia M. A. Gomes<sup>1</sup>; Marcelo M. Marinho<sup>2</sup>; Sandra M. F. O. Azevedo<sup>1</sup>**

<sup>1</sup>IBCCF – Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Brasil, <sup>2</sup>IBRAG – Instituto de Biologia Roberto Alcântara Gomes, Universidade do Estado do Rio de Janeiro, Brasil

Blooms of *Cylindrospermopsis* have become more and more frequent in Brazilian aquatic systems because of its competitiveness in tropical eutrophic systems. Beyond of ecological effects of the blooms, this genus is a potential producer of toxins (cylindrospermopsin/hepatotoxin and saxitoxin/neurotoxin), which cause problems to public health and environmental hazards. *Cylindrospermopsis raciborskii* is usually described as invasive specie and can represent up to 100% of total algal biomass under certain environmental conditions. This occurrence in tropical systems is usually associated to low light availability and its high affinity for nutrients. In order to evaluate these generalizations, we analyzed limnological data of 51 different Brazilian aquatic ecosystems, which *Cylindrospermopsis* occurs. The data base included limnological information of reservoirs and coastal lagoons, from the northeast, south and southeast regions of the country, comprising a latitudinal gradient. Nutrient concentration showed widely range from oligotrophic to hypereutrophic conditions. In general these systems presented low water transparency (Secchi 0,05 to 1,7m) and the annual average temperature is higher than 22°C. Relative contribution of *Cylindrospermopsis* in these systems ranged from 1 to 99% of phytoplankton biomass. The data analysis showed that relative contributions of *Cylindrospermopsis* greater than 80% were associated with high values of temperature, pH, alkalinity and conductivity. In general, low DIN concentrations were also related to high percentage contribution. So the success of *Cylindrospermopsis* in Brazilian aquatic ecosystems can be related to its low light requirements, high affinity for ammonium and its ability to tolerate high ionic concentration. Moreover, some of these systems have high ion concentrations which reflect in elevated conductivity values. Considering that some studies have pointed to the influence of some ions on the toxin production and that only Brazilian strains are known to produce saxitoxins we also discuss the possible relationships between the environmental factors and toxin production.

### P18: Seasonal community composition of microcystin-producing cyanobacteria in eutrophic Lake Tuusulanjärvi

**Hanna Sipari, Anne Rantala-Ylinen, Jouni Jokela, Matti Wahlsten and Kaarina Sivonen**

Department of Food and Environmental Sciences, Division of Microbiology, Viikki Biocenter 1, Viikinkaari 9, P.O. Box 56, 00014 University of Helsinki, Finland; hanna.sipari@helsinki.fi

Cyanobacterial mass occurrences (blooms) are common throughout the world and are frequently toxic posing a health risk for humans and animals. In Finnish lakes, the most prevalent cyanobacterial toxins are hepatotoxic microcystins, produced by the strains of *Microcystis*, *Anabaena* and *Planktothrix*. Microcystins are synthesized nonribosomally by an enzyme complex encoded by microcystin synthetase (*mcy*) gene cluster. These biosynthetic genes are widely used to detect and identify the toxin producers by molecular biology methods. The potential microcystin-producing cyanobacteria populations were followed in the eutrophic Lake Tuusulanjärvi in a two-year survey 2000-2002 and summers of 2006, 2008 and 2009. The changes in the quantity of *Anabaena*- and *Microcystis*- *mcyE* genes were determined with quantitative real-time PCR (qPCR). A chip assay was used to simultaneous detection of all potential microcystin producers. Microcystin variants were identified and quantified with LC-MS. In addition, RNA-based chip assay was developed to detect active microcystin producers. *Microcystis*-*mcyE* genes were found throughout the sampling period, while *Anabaena*-*mcyE* became prevalent later. In 2001, the *mcyE* gene copy numbers correlated well with microcystin quantities but similar trend was not found in 2000. The main microcystin variants were microcystin-LR in 2000 and demethylated-microcystin-RR in 2001. This survey showed that the same potential microcystin-producing cyanobacteria were present in Lake Tuusulanjärvi annually. Both potential microcystin producers *Microcystis* and *Anabaena* were present in the lake also in years 2006, 2008 and 2009. In 2008 and 2009 also *Planktothrix* *mcyE*-gene was detected by qPCR. The chip assay offered a high-throughput method for the simultaneous detection of all potential microcystin producers. Analysis of *mcyE* transcripts in 2006 samples confirmed that microcystins were produced by both *Anabaena* and *Microcystis*. To get a more comprehensive view on the succession of potential microcystin-producers in the lake, the effect of environmental parameters on toxic bloom development will be investigated.

## P19: Differentiation of akinetes in genus *Anabaena*

**Kaisa Haukka<sup>1</sup>, Sanni Jäppinen<sup>1</sup>, Sanna Suikkanen<sup>2</sup>, Kaisa Rantasärkkä<sup>1</sup>, Hao Wang<sup>1</sup> and Kaarina Sivonen<sup>1</sup>**

<sup>1</sup>Department of Food and Environmental Sciences, University of Helsinki, Finland; <sup>2</sup>Finnish Environment Institute, Finland

Planktonic cyanobacteria of genus *Anabaena* commonly form toxic blooms towards the end of summer in the Finnish aquatic environments. *Anabaena* strains can produce akinetes, or resting cells, which typically develop one cell away from a heterocyst during the late exponential phase of growth. Akinetes are thought to play an important role in overwintering of *Anabaena* and some other filamentous cyanobacteria. In the Baltic Sea, the viable seed bank size was found to range from 205 to 1913 akinetes g<sup>-1</sup> (ww) of sediment [1]. We have recently initiated genome, transcriptome and proteome level studies in order to identify the genes and proteins involved in akinete formation and germination. Our model strain *Anabaena* 1TU33s10, isolated from Finnish Lake Tuusulanjärvi, has been subjected to whole-genome sequencing. Concurrently, we have been optimizing akinete production, collection and storage methods. Conventional and quantitative PCR techniques targeting akinete-specific genes, such as *avaK* [2], will be used to follow the occurrence and expression of genes involved in cell differentiation at different stages of the life cycle. These methods will be applied to study *Anabaena* strains found in our culture collection and grown in different culture conditions and also in the Baltic Sea samples. Proteomics combined with the whole genome analysis will be used to identify new akinete differentiation and germination related genes. Our study will amend our understanding on the cyanobacterial life cycle strategies at the cellular level. Furthermore, the results will help in developing management systems for controlling the bloom-forming cyanobacteria, since akinetes may form a long-lasting storage of cyanobacteria in natural water ecosystems.

[1] Suikkanen S, Kaartokallio H, Hällfors S, Huttunen M, Laamanen M. 2010. Life cycle strategies of bloom-forming, filamentous cyanobacteria in the Baltic Sea. *Deep-Sea Res. II*, 57, 199-209; [2] Zhou R, Wolk CP. 2002. Identification of an akinete marker gene in *Anabaena variabilis*. *J. Bacteriol.* 184, 2529-2532.

## P20: New insights into bacteriochlorophyll e biosynthesis: a *bchU* mutant of *Chlorobaculum limnaeum* is viable and synthesizes bacteriochlorophyll f in its chlorosomes

**Kajetan Vogl<sup>1</sup>, Marcus Tank<sup>1</sup>, Zhenfeng Liu<sup>1</sup> and Donald A. Bryant<sup>1, 2</sup>**

<sup>1</sup>Dept. of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA; <sup>2</sup>Dept. Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717, USA

The *Chlorobaculum limnaeum* DSM1677T is a freshwater green sulfur bacterium that is brown-colored due to the presence of bacteriochlorophyll (BChl) e and isorenieratene in its chlorosomes. Chlorosomes are the most efficient light harvesting antenna complexes for phototrophic growth known and are found in the phototrophic members of the eubacterial phyla: *Chlorobi*, *Chloroflexi* and *Acidobacteria*. Due to its similarity to BChl c, BChl e also requires a C-20 methyltransferase in one of the last biosynthetic steps. The gene for this enzyme was identified in the recently sequenced 2.6-Mbp genome of *C. limnaeum*. We have established a gene inactivation system based on conjugative delivery of non-replicating plasmids of *Escherichia coli* for the inactivation of genes in *C. limnaeum*. Inactivation of the *bchU* gene, which encodes the C-20 methyltransferase, created a viable *C. limnaeum* mutant containing BChl f in its chlorosomes. Although BChl f has not yet been detected in nature, cultures of this mutant are greenish-brown in color and grow well. The BChl f aggregates of chlorosomes in whole cells absorbed maximally at 458, 486 and 707 nm (compared to 459, 527, and 722 nm for BChl e). The half-band width and the apparent molar extinction coefficient of the BChl f aggregates in the chlorosomes were smaller than those of BChl e in wild type chlorosomes, which suggests that these two pigments may form different suprastructures in the chlorosomes. RP-HPLC analyses showed that the BChl f homologs eluted approximately 1 minute earlier than the corresponding BChl e homologs of the wild type; the in-line absorption maxima were 461/641 nm and 471/656 nm, respectively. An intriguing observation was that the carotenoid contents and compositions of the two strains were different. The *bchU* mutant cells produced very small amounts of isorenieratene and instead produced greatly increased amounts of mainly neurosporene derivatives. This suggests that these cells may have a mechanism to sense and alter their carotenoid composition in response to changes in light harvesting. Growth experiments at different light intensities showed that the BChl f-producing strain grew with the same growth rate as the wild-type at high irradiance values, but grew much more slowly than the wild type at low irradiance values. Detailed analyses of the suprastructures of the chlorosomes and energy transfer properties of both strains will probably explain why BChl f is not naturally produced and used by green sulfur bacteria.

## P21: Photoautotrophic growth of *Chloroflexus aggregans* in an enrichment culture derived from hot spring microbial mats

Satomi Iwata<sup>1</sup>, Hiroyo Otaki<sup>1</sup>, Satoshi Hanada<sup>1, 2</sup>, Katsumi Matsuura<sup>1</sup>, Shin Haruta<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Tokyo Metropolitan University, Tokyo, JAPAN; <sup>2</sup>Bio-production Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, JAPAN

**Introduction:** *Chloroflexus aggregans* widely distributed in hot springs is a green filamentous photosynthetic bacterium. This bacterium has been shown to grow heterotrophically by anaerobic photosynthesis or by aerobic respiration [1]. *C. aggregans* MD-66<sup>T</sup> and YI-9 did not grow photoautotrophically when sulfide or thiosulfate is supplied as an electron donor [1], although genome analysis indicates *C. aggregans* MD-66<sup>T</sup> possesses a carbon dioxide fixing pathway, 3-hydroxypropionate pathway [2]. In *ex situ* incubation of microbial mats dominated by *C. aggregans*, Kubo *et al.* reported that this bacterium anaerobically oxidized sulfide in the light and this sulfide oxidation was promoted by supplemental addition of carbon dioxide [3]. Their findings suggested that *C. aggregans* in the mats utilized sulfide as an electron donor for CO<sub>2</sub> fixation. In this study, we tried to examine the photoautotrophy of *C. aggregans* collected from hot spring microbial mats.

**Results and Discussion:** Microbial mats at 65°C were collected from Nakabusa hot spring, Japan [4, 5]. A piece of the mats was cultivated in a medium containing NaHCO<sub>3</sub> as a sole carbon source under anaerobic conditions in the light. When sulfide and hydrogen were supplemented with the cultivation medium, bacterial growth was observed. The growth was even observed after 20 times of subcultures. Microscopic observation and genetic analysis based on 16S rRNA gene indicated that *C. aggregans* was dominant in the enrichment culture. Significant increase in bacteriochlorophyll *c* during the cultivation was observed spectroscopically to detect the growth of *C. aggregans* in the medium. *C. aggregans* also grew in an enrichment culture when sulfide was added as a sole electron donor. Microscopic observation indicated sulfide was oxidized to sulfur globules outside of *C. aggregans* cells. Hydrogen also worked as a sole electron donor for the autotrophic growth.

After *C. aggregans* was isolated, it did not grow autotrophically under the conditions described above. Autotrophy of *C. aggregans* may require the coexistence of other bacteria, e.g., fermenters which were found in the enrichment culture even after more than 20 times of subcultures.

[1] Hanada *et al.* (1995) Int. J. Syst. Bacteriol., 45, 676-681; [2] Klatt *et al.* (2003) Environ. Microbiol., 9, 2067-2078; [3] Kubo *et al.* (2011) Syst. Appl. Microbiol., 34, 293-302; [4] Otaki *et al.* (2012) Microbes Environ. in press; [5] Everroad *et al.* (2012) Microbes Environ. in press

## P22: Effects of environmental factors on production of bioactive peptides in *Nodularia spumigena* KAC 66

Shaista Hameed, Linda A. Lawton, and Christine Edwards

IDEAS - Institute for Innovation, Design and Sustainability Research, The Robert Gordon University, Aberdeen, United Kingdom

Due to heavy nutrient load, the Baltic Sea is *Nodularia spumigena* under the influence of eutrophication, which has resulted in the occurrence of heavy toxic algal blooms. In late summer the dominant and toxic strain *N. spumigena* along with the non-toxic *Aphanazomenon flos-aquae* and *Anabaena* spp., produces massive and lethal blooms in many areas of the Baltic Sea. *N. spumigena* has also been reported to have lethal blooms in Lake Alexandrina, Australia. As well as producing nodularins, *Nodularia* sp. also produces a range of other bioactive peptides such as spumigins and nodulopeptins, all of which have unclear function.

We recently characterized three new nodulopeptins (899, 901, 917) from *N. spumigena* KAC 66. Nodulopeptin 901 [1] demonstrated weak inhibition of protein phosphatase 1 (IC<sub>50</sub> 25 µg/ml). To gain further insight on the effects of environmental stress on growth and production of bioactive metabolites in *N. spumigena* KAC 66, a range of parameters were investigated which included; temperature, salinity, nitrate and phosphorus. Growth was monitored by cell biomass and chlorophyll-*a*. Intracellular and extracellular peptides were monitored by high performance liquid chromatography with photodiode array and mass spectrometry (HPLC-PDA-MS).

In common with many studies, the maximum amount of nodularin was retained within the cells during the seven week growth experiment. In contrast, as much as 40% of nodulopeptin 901 was detected in the growth media throughout the duration of experiments.

Temperature had the greatest effect on peptide production. Whilst growth was similar at 22°C, 25°C and 30°C, increase in temperature had a profound effect on nodularin production in that an increase from 22°C to 25°C resulted in a 50% decrease in intracellular nodularin levels. At 30°C little or no nodularin was detected. In contrast, whilst concentrations of nodulopeptin 901 decreased with increasing temperature, they were still detected at consistent levels suggesting they play an important role.

This is the first study to evaluate the effects of selected environmental parameters on nodularin/nodulopeptin production which ultimately may be helpful to explain the distribution, control of natural blooms and toxin levels of *N. spumigena* in the Baltic Sea and as well as laboratory based experiments.

[1] Schumacher, M. *et al.*, 2012. New nodulopeptins from *Nodularia spumigena* KAC 66. *Tetrahedron* 68(5), pp. 1622-1628.

## P23: Cyanobacterial biofilms on interior walls of natural draught cooling towers

Tomáš Hauer<sup>1,2</sup>, and Petra Böhmová<sup>2</sup>

<sup>1</sup>Centre for Phycology, Institute of Botany AS CR, Třeboň, Czech Republic; <sup>2</sup>Department of Botany, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Cooling towers are a widely used way of waste heat dissipation in both industry and power generation. As they are open systems, they offer a niche for broad spectrum of organisms from surrounding environment. Our study was focused on biofilms formed mostly by cyanobacteria, accompanied with algae and mosses, occurring in the upper part of the towers. These biofilms are contributing to the tower construction damage, especially to the degradation of the protective painting by metabolic activities. They also contribute to the reduction of cooling power by clogging the cooling fill in the tower. Samples for analyses were taken from 16 towers in seven plants. Altogether 33 cyanobacterial taxa were found. On four towers the community composition in relation to the geographic exposition was tested. The biofilms exhibit composition heterogeneity according to their sun light exposure. Members of the genus *Leptolyngbya* prefer and dominate north facing walls, on the other hand, *Gloeocapsa* and several scytonemin producing taxa dominate the walls, which are exposed to the direct sun light for at least a small part of the day. Thus, under constant water and nutrient regime the biofilm composition is influenced by light conditions. Upper parts of towers with integrated combustion products outlet are completely without phototrophs.

## P24: Isolation and characterization of the unicellular diazotrophic cyanobacterium Group C TW3 from the tropical western Pacific Ocean

Yukiko Taniuchi<sup>1</sup>, Yuh-ling Lee Chen<sup>1,3</sup>, Hsung-Yung Chen<sup>2,3</sup>, Mei-Ling Tsai<sup>4</sup>, Kaori Ohki<sup>5</sup>

<sup>1</sup>Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 80424 Taiwan; <sup>2</sup>Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung 80424 Taiwan; <sup>3</sup>Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 80424 Taiwan; <sup>4</sup>Department of Sea Food Sciences, National Kaohsiung Marine University, Kaohsiung 81157 Taiwan; <sup>5</sup>Department of Marine Resources, Fukui Prefectural University, 1-1 Gakuen-cho, Obama-shi, Fukui, 910-0001, Japan

Biological nitrogen (N) fixation is an important process in the marine N cycle, especially in oligotrophic oceans. Recent molecular approaches have revealed an abundance of diazotrophic unicellular cyanobacteria and proteobacteria, which may contribute to the marine N cycle. Unicellular diazotrophic cyanobacteria are divided into Groups A, B, and C by the phylogenetic analysis of *nifH* gene. Isolations and physiological studies of unicellular cyanobacteria from the oceanic environment were only reported in *Crocospaera watsonii* WH8501 which belongs Group B. Isolation of Group A have not succeeded. Pertaining to Group C, *Cyanothece* sp. ATCC51142 isolated from intertidal sands, belongs to Group C. Attempts to isolate other Group C cells from the open ocean have been unsuccessful. Consequently, physiological studies on open ocean unicellular diazotrophic cyanobacteria have been restricted to Group B. Successful isolation of any Group A or oceanic strains of Group C would render studies of important physiological characteristics possible, which will greatly expand our understanding of the relative roles of the three groups in the N cycle of open oceans.

The Kuroshio Current is a N-deficient oligotrophic current in the western Pacific. Its surface nitrate + nitrite concentrations were  $16 \pm 4 \text{ nmol l}^{-1}$  in the summer and  $107 \pm 84 \text{ nmol l}^{-1}$  in the winter (Chen *et al.*, 2008). Chen *et al.* (2009) reported that the densities of unicellular cyanobacteria in the upstream Kuroshio surface water near Taiwan Island to be  $43.07 \pm 18.6 \times 10^2 \text{ cells L}^{-1}$  in the 2.5-5  $\mu\text{m}$  size fraction and  $14.1 \pm 28.0 \times 10^2 \text{ cells L}^{-1}$  in the 5-10  $\mu\text{m}$  fraction.

Here we report isolation of the unicellular cyanobacterium TW3 collected from the surface water of the upstream Kuroshio. TW3 grew in nitrogen-deficient artificial seawater. Cells of TW3 are green rod with a dimension of 2.5-3.0  $\mu\text{m}$  in width and 4.0-6.0  $\mu\text{m}$  in length. Phylogenetic analyses of both 16S rRNA and *nifH* gene fragments indicated that TW3 had more than 98% identities to previously isolated *Cyanothece* sp. ATCC51142 and *Gloeocapsa* sp., suggesting that TW3 belongs to Group C. In addition, both TW3 and *Cyanothece* sp. ATCC51142 share the same morphological characteristics of being sheathless and rod-shaped, having binary fission in a single plane, and possessing dispersed thylakoids. TW3 grew aerobically in N-deficient artificial seawater, and showed the highest growth rate of  $0.035 \text{ h}^{-1}$  when cultured at 30°C and  $140 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . Its N<sub>2</sub> fixation rate, when optimally grown in a 12h/12h light-dark cycle, was  $7.31 \times 10^{-15} \text{ mol N cell}^{-1} \text{ d}^{-1}$ . Immunocytochemical staining of nitrogenase showed the existence of diazotrophic cells sharing similar morphological characteristics of TW3 in the Kuroshio water from which it was isolated.

Chen *et al.* (2008) *Limnol Oceanogr* 53: 1705-1721; Chen *et al.* (2009) *Mar Ecol Prog Ser* 385: 111-126

## P25: Embryotoxicity assessment of a *Microcystis* extract with *Daphnia* *in vitro* egg test

Piedade Barros<sup>1\*</sup>, Rosário Martins<sup>1, 2</sup>

<sup>1</sup>CISA - Centro de Investigação em Saúde e Ambiente, ESTSP - Escola Superior de Tecnologia da Saúde do Porto, IPP - Instituto Politécnico do Porto, Portugal. \*pgb@estsp.ipp.pt; <sup>2</sup>CIIMAR/CIMAR, Center for Marine and Environmental Research, University of Porto, Rua dos Bragas 289, Porto, Portugal.

*Microcystis aeruginosa* is a colonial unicellular cyanobacteria, usually found in eutrophic and hipereutrophic waters. Microcystins are the main toxins produced. As chronic toxicity test for daphnid is time consuming, the developmental stages of *Daphnia* eggs *in vitro* has been used in several works as an alternative to the 21 days reproduction test [1,2,3]. The sensitivity of different species of *Daphnia* to cyanobacteria varies depending on the species and strain and on the mode of exposure. Eggs are an early undifferentiated stage of development and may be a way to compare the differences of sensitivity between species. The main aim of this study was to assess the effects of a *Microcystis aeruginosa* extract on the *in vitro* survival and development of the parthenogenetic eggs of *D. pulex*, *D. pulicaria* and *D. magna*. The parthenogenetic eggs used in this study were obtained from adult females. At the beginning of the test eggs were at stage I and II in their development and were removed from the female body by applying gentle pressure to the posterior region of the brood chamber with a dissecting needle. Extruded eggs were collected in M4 medium. Tests were performed in 50 ml glass flasks and 10 eggs were exposed for each extract concentration. Eggs were incubated at 20±1 °C with 16 h photoperiod and were examined microscopically every 24 h during the test period. We examined embryos for stage of development and recorded mortality. EC50 values were determined by the probit method. In all the concentrations tested there was arrested development in embryos, EC50 (mg/ml) for the three species of daphnia were only slightly different. *Daphnia magna* was the most resistant specie with EC50 0,34 (mg/mL). As EC50 values for egg *in vitro* development were lower than CL50 values for neonates and adults survival from the same species, we consider this test as an alternative test assessment for cyanobacteria toxicity in daphnia development.

[1] Obreshkova, V., Fraser, A.W., 1940. Growth and differentiation of *Daphnia magna* eggs *in vitro*. Biol. Bull. 78, 428-436; [2] Ohta, T., Tokishita, S., Shiga, Y., Hanazato, T., Yamagata, H., 1998. An assay system for detecting environmental toxicants with cultured cladoceran eggs *in vitro*: malformations induced by ethylenethiourea. Environ. Res. 77, 43-48; [3] Sobral, O., Chastinet, C., Nogueira, A., Soares, A., Goncalves, F., Ribeiro, R., 2001. *In vitro* development of parthenogenetic eggs: a fast ecotoxicity test with *Daphnia magna*? Ecotoxicol. Environ. Saf. 50, 174-179.

## P26: Are the cyanobacteria inhibiting biological sand crust indeed high light organisms?

Hagai Ra'anan<sup>1</sup>, Danny Ionescu<sup>2</sup>, Itzhak Ohad<sup>1</sup>, Nir Keren<sup>1</sup> and Aaron Kaplan<sup>1</sup>

<sup>1</sup>Plant & Environmental Sciences, Hebrew University of Jerusalem, Israel; <sup>2</sup>Microsensor Group, Max Planck Institute for Marine Microbiology, Bremen, Germany

Biological soil crusts play an important role in stabilizing sandy areas and can influence the biotic composition of deserts. Destruction of these crusts is considered an important promoter of desertification in arid and semi-arid regions. The crusts are formed by the adhesion of the sand to extracellular polysaccharides (EPS) secreted mostly by filamentous cyanobacteria (including *Nostoc*, *Microcoleus*, *Chroococcidiopsis* and *Gloeocapsa*). These organisms, the main primary producers in biological desert crusts, are able to acclimate to extreme temperatures, frequent hydration/dehydration cycles and high light intensity. The mechanisms involved, however, are largely unknown. It is likely that the ability to activate metabolism and grow when water is available and to shutdown metabolic activities during dehydration plays an important role in this acclimation.

One important example is the photosynthetic activity: It is well-established that photosystem II (PSII) is highly susceptible to photoinhibition due to rapid degradation of core proteins. Coordination of light energy flux to the reaction centers with the rate of electron transport and CO<sub>2</sub> fixation is extremely important, particularly during dehydration; otherwise, photodynamic damage of the photosynthetic machinery may occur.

To uncover protective mechanisms from excess light during desiccation, we compared the response to excess light of several desiccation-tolerant cyanobacteria with well-studied model freshwater cyanobacteria. Measurements of oxygen evolution and fluorescence parameters were taken on crusts and isolated strains from the sand dunes of Nizzana, NW Negev, grown on sand or liquid media.

Surprisingly our results showed much less resistance of the desiccation-tolerant cyanobacteria to excess light as compared to model cyanobacteria. Further, the desiccation-tolerant cyanobacteria within the crusts showed a rapid decline in fluorescence yield already at light intensities 1/10 of full sunlight and a faster QA re-oxidation after high light treatment.

Use of microelectrode for oxygen, pH and light penetration showed that maximum oxygen evolution occur at very low light levels. Apparently, the structure of the crusts and reflection of light therein leads to the formation of "light pockets" beneath the "photot zone" enabling the photosynthetic production of oxygen in this depth.

These results and those to be presented suggest that photosynthetic activity of the cyanobacteria in the crust mostly occurs at low light levels and that protection mechanisms are activated as soon as direct sunlight hits the crust. This protection mechanism may involve a cyclic electron flow within PSII.



## P27: Impact of the large Alqueva dam on cyanobacteria in the Guadiana estuary, Southern Iberia

Helena M. Galvão, Rita B. Domingues, Ana B. Barbosa, and Cátia C. Guerra

Center for Marine and Environmental Research, Universidade do Algarve, Gambelas, 8005-139 Faro, PORTUGAL

The Guadiana River, running along the Southern border between Portugal and Spain has the fourth largest drainage basin of Iberian rivers (67 840 km<sup>2</sup>), but a series of dams, have severely restricted its freshwater flow (ca. 75 %), and the recent construction of the large Alqueva dam increased flow regulation up to 81% of the total catchment area (55 000 km<sup>2</sup>) starting in 2003.

Cyanobacteria blooms have been reported in the Guadiana River in association with seasons and/or years of low freshwater flow. Microbial ecology studies carried out from 1996 to 1998 showed a well defined chlorophyll maximum in the upper estuary. Changes in freshwater flow lead to alterations in water quality and hydrography, thus affecting phytoplankton composition and succession. River flow after completion of the Alqueva dam was severely restricted even during winter months with high rainfall. During the period of dam construction (1999-2000), the sediment load transported downstream increased dramatically causing severe photolimitation for the phytoplankton which resulted in the absence of blooms and very low chlorophyll values throughout spring and summer. Afterwards, during dam filling (2002-2003), nutrient concentrations increased, as well as cyanobacteria abundance, while diatom abundance remained. After this period, total phytoplankton abundance and succession followed the typical trend observed before dam filling. However, specific diversity and chlorophyll concentration tended to decrease after 2002. In particular, cyanobacteria populations decreased remarkably and potentially toxic species virtually disappeared. Thus, our prediction of increased eutrophication with more frequent cyanobacteria blooms in the river Guadiana following dam construction was not fulfilled, instead an oligotrophication trend is now being observed with diminishing chlorophyll, phytoplankton (incl. cyanobacteria) abundance and diversity. Further studies should reveal if this trend is long-term or only a transitional phase.

## P28: Water quality and toxicity assessment of Cyanobacterial strains in three tunisian reservoirs Bir M'cherga (semi-arid area), Nabhana and Sidi Saâd (arid area).

Amel Ben Rejeb Jenhani<sup>1</sup>, Afef Fathalli<sup>1</sup>, Cristiana Moreira<sup>2</sup>, Agostinho Antunes<sup>2</sup>, Martin Welker<sup>3</sup>, Mohamed S. Romdhane<sup>1</sup>, Vítor Vasconcelos<sup>2,4</sup>

<sup>1</sup>Unité de Recherche Ecosystèmes et Ressources Aquatiques, Institut National Agronomique de Tunisie, Tunisia; <sup>2</sup>Centro de Investigação Marinha e Ambiental- CIIMAR/CIMAR, Universidade do Porto. Porto, Portugal; <sup>3</sup>Technische Universität Berlin, Berlin, Germany; <sup>4</sup>Departamento de Biologia. Faculdade de Ciências, Universidade do Porto, Portugal

Eutrophication has been identified as an environmental problem in many aquatic ecosystems. Resulting from nutrient enrichment and the increase in aquatic plant production, this phenomenon usually leads to a massive development of phytoplankton, often dominated by cyanobacteria [1]. In freshwater ecosystems, the cyanobacteria proliferation, favored by certain environmental conditions, may pose a significant threat to environment and public health, because of their metabolites known to cause adverse effects in diverse organisms including plants, mammals, birds, fish and other aquatic organisms [2-4]. Therefore, it is very important to identify and characterize toxin-producing strains of cyanobacterial populations in field samples. With this mind, a study of environmental conditions, the species composition of phytoplankton including cyanobacteria and their toxicity assessment were performed in three Tunisian dams located in semi-arid and arid region of the country: Bir M'cherga, Nabhana and Sidi Saad.

Cyanobacteria in these water bodies were represented by 18 species, eight of which are cited in the literature as potentially toxic. The evaluation of the toxic potential of 15 isolates belonging to *Cylindrospermopsis raciborskii* species, *Microcystis aeruginosa* and *Planktothrix agardhii*, was performed by molecular biology tools. The results revealed that all the isolated strains were not producing any PCR amplicon with the primers used for neither cylindrospermopsin nor saxitoxin. Only a *Microcystis aeruginosa* strain isolated from Nabhana could produce the hepatotoxin microcystin (MC). Indeed, it expressed the six characteristic segments of the microcystin synthetase *mcy* cluster (*mcyA*, -B, -C, -D, -E and -G). This was confirmed by MALDI-TOF analysis which revealed the presence of two microcystin variants: MC-LR and MC-YR. Taxonomic identification of strains was verified by amplification and sequencing of 16S rRNA gene fragment. The phylogenetic analysis based on 16S rRNA and ITS sequences and the maximum Likelihood method in PAUP\* 4.0b10, showed the high genotype similarity of investigated Tunisian *Microcystis* strains compared to those available in the GenBank database.

[1] Dauta A., Feuillade., (1995). Croissance et dynamique des populations algales. IN Pourriot R. et Meybeck M. (eds). Limnologie générale. Masson, Paris Milan Barcelone. Collection Ecologie. pp 328 – 350; [2] Chorus I., Bartram J., (1999). Toxic Cyanobacteria in water. A guide to their public Health consequences, monitoring and management, WHO Ed. E & FN SPON, 416 pp.; [3] Saqrane S., Oudra B. (2009). CyanoHAB occurrence and water irrigation cyanotoxin contamination: ecological impacts and potential health risks. Toxins; 1: 113-122.; [4] Vasconcelos V.M. (1999). Cyanobacterial toxins in Portugal: effects on aquatic animals and risk for human health. Brazilian Journal of Medical and Biological Research; 32: 249-254.



# **POSTER>SESSION III**

## **PHYSIOLOGY, METABOLISM, SENSING AND SIGNAL TRANSFER**



## POSTERS> III. PHYSIOLOGY, METABOLISM, SENSING AND SIGNAL TRANSFER

### P29: The role of phycobilisome degradation in the acclimation of cyanobacteria to nitrogen limitation

**Eitan Salomon, Leeat Bar-Eyal and Nir Keren**

Department of Plant & Environmental Sciences, The Alexander Silberman Institute of Life Sciences, The Hebrew University of Jerusalem, Israel

Nitrogen limitation forces photosynthetic organisms to reallocate intracellular nitrogen towards essential processes. At the same time, nitrogen limitation increases the probability of photo-damage. Decrease in the rate of energy-demanding metabolic processes, downstream of the photosynthetic apparatus, can result in over-reduction of photosynthetic electron carriers and consequently in photoinhibition. Non-diazotrophic cyanobacteria cope with this situation by reducing the size of their phycobilisome antenna (PBS) and by modifying their photosynthetic apparatus. These changes can serve two purposes, to provide extra amino-acids and to reduce excitation pressure. In this work we studied which process - nitrogen recycling or photo-protection, is the more prominent determinant for PBS degradation. We took a detailed look at the response of the photosynthetic apparatus to nitrogen limitation under two experimental conditions where electron flow rates were low. In the first setup, *Synechocystis* sp. strain PCC 6803 cells were grown under growth light and low light intensities (GL = 60 and LL = 6  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively). In the second setup, prior to the nitrogen step down process, the cells were allowed to acclimate to Mn limitation (Mn<sup>-</sup>), reducing the activity of both photosystems (Salomon and Keren 2011).

Absorption spectra measurements indicated that nitrogen limited *Synechocystis* 6803 cells that were grown under a low light intensity or were Mn<sup>-</sup> pre-acclimated, displayed slower PBS degradation kinetics than those grown under growth light intensities. Time resolved absorption spectroscopy measurements of P<sub>700</sub> activity demonstrated that low light and Mn<sup>-</sup> cultures retained higher activity of both photosystems, as compared to growth light cultures. The total protein content of both photosystems was reduced under N<sup>-</sup> conditions, as visualized by western blots. The protein content of PSII proteins was even lower under growth light intensities. These results suggest that the most prominent determinant for the rapid disassembly of PBS is the need to reduce the light absorption cross section in order to avoid photo-damage during the acclimation process, when electron acceptors are scarce. In light of these results it is interesting to reexamine our understanding of photo-inhibitory processes. In their native environments, cyanobacteria often encounter nutrient limitations under low to moderate light intensities, similar to the conditions tested here. This is a much more frequent situation than dealing with super-saturating light intensities under nutrient sufficient conditions forced upon photosynthetic organisms in many photo-inhibition experiments. In environmental terms, the low light intensities used here (up to 2% of the maximal solar irradiation flux) are sufficient for imposing changes in the organization of the photosynthetic under what are very prevalent conditions in nature.

### P30: Probing *Rhodobacter capsulatus* AmtB function with site-directed mutagenesis

**Zahra Choolaei, Imene Abdelmadjid, Narimane Boukharouba, Anh Kiet Nguyen and Patrick C. Hallenbeck**

Département de microbiology et immunology, Université de Montréal, CP 6128, succursale Centre-ville, Montréal, PQ, H3C 3J7 Canada

Amt proteins are found in almost all forms of life; bacteria (Amt, ammonium transporter), yeast (Mep, methylamine permease protein) and animals Rh (rhesus factor) (1). AmtB is a homotrimeric cytoplasmic membrane protein with a hydrophobic pore. Conduction of external NH<sub>4</sub><sup>+</sup> requires deprotonation at the exterior, transfer of NH<sub>3</sub> through the cytoplasmic membrane, and reprotonation at the AmtB cytoplasmic surface. The role of AmtB in many bacteria is unclear since passive diffusion of NH<sub>3</sub> permits growth on ammonium except at low pHs and very low ammonium concentrations. However, in some purple non-sulfur photosynthetic bacteria AmtB has been shown to be essential for ammonium switch-off of nitrogenase activity. In some bacteria AmtB is thought to regulate nitrogenase activity by the ammonium induced sequestration of GlnK and DraG to the membrane (3, 4). However, complex formation, at least in some AmtB variants, is not sufficient (4).

*Rhodobacter capsulatus* possesses two genes coding for ammonium transport proteins, *amtB* and *amtY*. A previous study showed that AmtB was solely responsible for nitrogenase regulation and an *amtY* mutant had no apparent phenotype, i.e. was normal for methylamine transport and nitrogenase switch-off. Thus, the function of AmtY was unclear. Here we show, by qPCR, that *amtY* is transcribed in *R. capsulatus* under N-limiting conditions, again raising questions as to its function. We cloned *amtY* and expressed it in an *amtB* *Escherichia coli* strain (GT1001). Surprisingly, AmtY appears functional in a number of respects in that species. When expressed in *E. coli* AmtY is active in methylammonium transport and can correct the growth defect on ammonium of the AmtB-strain. However, although RcAmtY is capable of binding EcGlnK, EcGlnK sequestration is unaltered in response to an NH<sub>4</sub><sup>+</sup> shock.

[1] Tremblay, P.L., and Hallenbeck, P.C. Of blood, brains and bacteria, the Amt/Rh transporter family: emerging role of Amt as a unique microbial sensor. *Mol. Microbiol.* 71: 12-22, 2009; [2] Yakunin, A.F. and Hallenbeck, P.C. AmtB is necessary for NH<sub>4</sub><sup>+</sup> induced nitrogenase switch-off and ADP-ribosylation in *Rhodobacter capsulatus*. *J. Bacteriol.* 184:4081-4088, 2002; [3] Tremblay, P.-L., Drepper, T., Masepohl, B., and Hallenbeck, P.C. Membrane sequestration of PII proteins and nitrogenase regulation in the photosynthetic bacterium *Rhodobacter capsulatus*. *J. Bacteriol.* 189:5850-5859, 2007; [4] Tremblay, P.-L., and Hallenbeck, P. C. "Ammonia-induced Formation of an AmtB-GlnK Complex is not sufficient for Nitrogenase Regulation in the photosynthetic bacterium *Rhodobacter capsulatus*. *J. Bacteriol.* 190:1588-1594, 2008

### P31: Exploring the transcriptome of *Anabaena*: a search for non-coding RNAs

Jan Mitschke<sup>1</sup>, Agustín Vioque<sup>2</sup>, Wolfgang R. Hess<sup>1</sup> and Alicia M. Muro-Pastor<sup>2</sup>

<sup>1</sup>University of Freiburg, Faculty of Biology, Freiburg, Germany; <sup>2</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Sevilla, Spain

Non-coding RNAs appear involved in the regulation of virtually every bacterial response to stress. This group of molecules includes small RNA species, that mostly act *in trans* at the post-transcriptional regulation of mRNAs (sRNAs) and RNAs that are transcribed from the complementary strand of annotated genes (antisense RNAs). We have begun a global approach to the identification of non-coding RNAs in heterocystous cyanobacteria, with a special interest on RNAs potentially involved in the regulation of responses to nitrogen stress, including heterocyst differentiation.

Our approach is based on a transcriptomic analysis carried out by deep sequencing of total RNA (RNA-Seq) isolated from cells that are subjected to nitrogen deficiency for 8 h (in comparison to control samples not subjected to nitrogen deficiency). By using a modified protocol designed to counter-select processed transcripts, we carried out a genome-wide identification of transcriptional start sites (TSS) throughout the chromosome and the plasmids of *Anabaena* sp. PCC 7120 (1). This dataset confirms most of the previously defined TSS for *Anabaena*, while identifying unique nitrogen-regulated TSS for the majority of genes previously defined as involved in heterocyst differentiation or adaptation to nitrogen stress. Because our RNA-Seq approach includes a *hetR* mutant strain, the identification of transcriptional changes observed in the wild-type strain but not in the *hetR* mutant defines a large group of transcriptional responses that seem to be specifically linked to the differentiation of heterocysts.

When analyzed in combination with computational predictions for Rho-independent transcriptional terminators (2), the TSS dataset allows the identification of putative sRNA species that are transcribed from intergenic regions located between two annotated genes. We are currently characterizing some of these sRNAs that exhibit a strong regulation of their expression in response to nitrogen availability, and therefore might correspond to functional non-coding RNAs involved in the regulation of the responses to nitrogen stress and in the regulation of heterocyst differentiation.

[1] Mitschke J., A. Vioque, F. Haas, W. R. Hess and A. M. Muro-Pastor (2011) Proc. Natl. Acad. Sci USA 108:20130-20135; [2] Ionescu D., B. Voss, A. Oren, W. R. Hess and A. M. Muro-Pastor (2010) J. Mol. Biol. 398:177-188.

### P32: Light induced movement of cyanobacterial cells

Sven De Causmaecker<sup>1</sup>, Philipp Savakis<sup>2</sup>, Nils Schürgers<sup>1</sup>, Veronika Angerer<sup>2</sup>, Lars Oliver Essen<sup>2</sup>, Annegret Wilde<sup>1</sup>

<sup>1</sup>Institute of Molecular Biology and Microbiology, Justus-Liebig University Giessen, Germany; <sup>2</sup>Department of Chemistry, Philipps-University Marburg, Germany

*Synechocystis* sp. PCC 6803 exhibits flagellar-independent "twitching motility" that allows bacteria to move over moist surfaces using type IV pili. Mutants that lost type IV pili are non-motile. In order to use optimal light qualities and quantities for photosynthesis, they are able of directed movement along a light gradient. Regulation of phototactic motility is complex and involves many different gene products, including amongst others different photoreceptors, the RNA chaperone Hfq and adenylate cyclases.  $\Delta hfq$  mutants are non-motile and most of their type IV pili were missing. Consequently, they have lost also their natural competence for transformation by exogenous DNA. Microarray experiments revealed several mRNAs and small regulatory RNAs belonging to the cAMP receptor protein regulon that are differentially expressed in *hfq* mutants. Expression analyses and co-immunoprecipitation experiments will be discussed in connection with RNA-based regulatory processes in *Synechocystis* sp. PCC 6803.

In addition, we demonstrate a biological function of the Cph2 photoreceptor in motility. Cph2, a bilin-binding photoreceptor protein from the cyanobacterium *Synechocystis* sp. PCC 6803 with multiple GAF domains, inhibits phototaxis towards blue light. We show that Cph2 perceives blue light by its third GAF domain. This GAF3 domain belongs to the cyanobacteriochrome family and undergoes photoconversion between green and blue-light absorbing states. The two N-terminal GAF domains support photoconversion between red and far-red absorbing states. Cph2 differs from most other phytochromes by harboring EAL and GGDEF domains, both associated with turnover of the second messenger c-di-GMP. Upon blue light irradiation the GAF3 domain together with the downstream GGDEF domain shows elevated c-di-GMP synthesis *in vitro* compared to green light irradiation. A photochemically inactive mutant protein exhibits light independent c-di-GMP production. Overexpression of the GAF-GGDEF module in

*Synechocystis* sp. PCC 6803 leads to blue-light dependent inhibition of motility, expression of the N-terminal Cph2 fragment harbouring the EAL domain as well as an *E. coli* EAL domain protein complement this phenotype. C-di-GMP has been shown to regulate flagellar and pili-based motility in several bacteria. Here we provide the first evidence that this second messenger is also involved in light dependent regulation of cyanobacterial phototaxis.

### P33: Towards understanding the role of ORF *alr4393* in heterocyst differentiation in *Anabaena* sp. PCC 7120

**Antonio López-Lozano, Enrique Flores, and Antonia Herrero**

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, E-41092 Sevilla, Spain

NtcA is a transcriptional regulator of widespread distribution in cyanobacteria, controlling the expression of most genes involved in nitrogen metabolism. In the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120, control by NtcA includes the regulation of heterocyst differentiation [1,2]. Downstream of *ntcA*, a conserved ORF is found in most of the cyanobacterial genomes sequenced to date, which in *Anabaena* sp. PCC 7120 is named *alr4393*. Although no function has been identified for this ORF, northern blot analysis performed with RNA isolated from ammonium-grown filaments subjected to nitrogen deprivation showed that the expression of *alr4393* is increased but delayed with regard to that of *ntcA*. Furthermore, in a mutant strain bearing the ORF promoter region and its first three codons fused to the *gfp-mut2* gene, the GFP protein was observed mostly localized to proheterocysts after transferring the cells to media lacking combined nitrogen. In order to study the function of the Alr4393 protein in *Anabaena* sp. PCC 7120, a mutant strain bearing an in-frame deletion within its ORF has been constructed and named CSAL2. The ability to grow using different nitrogen sources was studied in solid and liquid media in this mutant in comparison to the wild type. Using NtcA is a transcriptional regulator of widespread distribution in cyanobacteria, controlling the expression of most genes involved in nitrogen metabolism. In the heterocyst-forming cyanobacterium *Anabaena* sp. PCC 7120, control by NtcA includes the regulation of heterocyst differentiation [1,2]. Downstream of *ntcA*, a conserved ORF is found in most of the cyanobacterial genomes sequenced to date, which in *Anabaena* sp. PCC 7120 is named *alr4393*. Although no function has been identified for this ORF, northern blot analysis performed with RNA isolated from ammonium-grown filaments subjected to nitrogen deprivation showed that the expression of *alr4393* is increased but delayed with regard to that of *ntcA*. Furthermore, in a mutant strain bearing the ORF promoter region and its first three codons fused to the *gfp-mut2* gene, the GFP protein was observed mostly localized to proheterocysts after transferring the cells to media lacking combined nitrogen. In order to study the function of the Alr4393 protein in *Anabaena* sp. PCC 7120, a mutant strain bearing an in-frame deletion within its ORF has been constructed and named CSAL2. The ability to grow using different nitrogen sources was studied in solid and liquid media in this mutant in comparison to the wild type. Using nitrate or ammonium, growth of strain CSAL2 was similar to that of strain PCC 7120. However, at the expenses of N<sub>2</sub>, growth of strain CSAL2 was severely impaired. Microscopic observation of diazotrophic cultures of this mutant showed that the frequency of heterocysts was considerably higher in the strain CSAL2 than in the wild type. Regarding heterocyst distribution, the number of cells between heterocysts was lower in the strain CSAL2 than in the wild type. Because *alr4393* was preferentially expressed in heterocysts and strain CSAL2 showed impaired diazotrophic growth, we are currently checking the expression of a number of genes involved in heterocyst maturation and function by northern blot analysis in this mutant strain. Besides, expression of *ntcA* and the gene encoding the differentiation-specific factor HetR has been studied using GFP as a reporter in the mutant background. A mutant strain overexpressing Alr4393 has been also generated and named strain CSAL21. The phenotypic characteristics of this mutant are currently being investigated and will be presented.

[1] Flores E. & Herrero A. (2010) Nature Reviews Microbiology 8: 39-50; [2] Herrero A. et al. (2004) FEMS Microbiology Reviews 28: 469-487.

### P34: Involvement of the transcriptional regulator CyAbrB in the central carbon metabolism in *Synechocystis* sp. PCC 6803 (II): Significance of 2-oxoglutarate in survival of the *cyAbrB* mutant under photomixotrophic conditions

**Ayumi Kizawa<sup>1</sup>, Yuki Kaniya<sup>1</sup>, Atsuko Miyagi<sup>2</sup>, Maki Kawai<sup>1,2</sup>, Hirofumi Uchimiya<sup>2</sup>, and Yukako Hihara<sup>1,2,3</sup>**

<sup>1</sup>Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University, Saitama, Japan; <sup>2</sup>Institute for Environmental Science and Technology, Saitama University, Saitama, Japan; <sup>3</sup>PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan

A gene-disrupted mutant of *cyabrB* (sll0822) in *Synechocystis* sp. PCC 6803 exhibited severe growth inhibition under photomixotrophic conditions. We performed metabolome analysis of the wild type and  $\Delta$ *cyabrB* cells under photoautotrophic and photomixotrophic conditions and found that amounts of pyruvate and 2-oxoglutarate (2-OG) in the mutant were significantly lower than those in the wild type irrespective of trophic conditions. In this report, the causes and effects of the shortage of these metabolites were further examined.

Time course change of transcript levels of *pyk* genes (sll0587, sll1275) encoding pyruvate kinase and *icd* gene (slr1289) encoding isocitrate dehydrogenase was examined in the wild type and  $\Delta$ *cyabrB* cells upon the shift to photomixotrophic conditions. These transcripts were up-regulated by the addition of glucose in the wild type, whereas those in the  $\Delta$ *cyabrB* mutant remained at low levels. Gel mobility shift assay showed binding of purified His-Sll0822 protein to the upstream regions of *pyk* and *icd* genes. These results suggest that CyAbrB works as a positive regulator for these genes, which are involved in the central carbon metabolism. We observed that growth inhibition of the  $\Delta$ *cyabrB* mutant under photomixotrophic conditions can be fully rescued by the addition of 2-OG to the growth medium and be partially rescued by pyruvate. The addition of glutamate could not improve the growth of the mutant. These results suggest that the operation of GOGAT reaction but not the resultant product is critical for survival of the  $\Delta$ *cyabrB* mutant under photomixotrophic conditions. To examine the effect of the addition of 2-OG to cellular metabolism, metabolome analysis of photomixotrophically grown  $\Delta$ *cyabrB* mutant with or without 2-OG was performed. Unexpectedly, the addition of 2-OG affected not only pathways located downstream of GS-GOGAT cycle, but also upstream metabolic processes such as glycolysis and the oxidative pentose phosphate pathway. The significance of 2-OG in photomixotrophic growth will be discussed.

### P35: Expression of the *psbA* and *hoxH* genes in *Synechococcus* sp. PCC 7002 under environmental stress conditions

**Bogdan Drugă<sup>1</sup>, Ciprian Chiș<sup>1,2</sup>, Iulia Simeoni<sup>1,2</sup>, Oana Sicora<sup>1,2</sup>, Cosmin Sicora<sup>1,2</sup>**

<sup>1</sup>Institute of Biological Research, Cluj-Napoca, Romania; <sup>2</sup>Centre of Biological Research, Jibou, Romania  
Corresponding authors: bogdan.druga@icbcluj.ro, cosmin.sicora@gmail.com

The structure of the photosynthetic machinery in cyanobacteria is highly conserved, as well as in green algae and higher plants. The core proteins of the photosystem (PS) II, D1 and D2, bind all the redox-active components involved in electron transfer of PSII. D1 protein is one of the main sites of damage by a wide variety of environmental factors, and it is encoded by the *psbA* genes, instead of higher plants which possess only one *psbA* gene. Various studies focused on transcriptional regulation of these genes have displayed two distinct regulation strategies to response to stress conditions. One is to use two distinct types of D1 protein, one, present under normal growth conditions is replaced by the other one under stress conditions. Another strategy is to enhance the production of the same type of D1 protein, compensating the higher rate of damage under stress. Recently a new type of D1 was identified (D1'), specifically induced under microaerobic conditions with possible implications in anaerobic hydrogen production.

In this study we analyzed the cyanobacterial strain *Synechococcus* sp. PCC 7002, in order to elucidate what D1 regulation strategy uses as a response to change in certain environmental factors. Moreover, the expression of the *hoxH* gene (encoding bidirectional hydrogenase) was assessed under the same conditions. In this strain, two *psbA* genes encode the same D1 protein, while another *psbA* gene encodes divergent D1 isoform. The standard growth conditions for this strain were: light irradiance of 50  $\mu\text{E} \times \text{m}^{-2} \times \text{s}^{-1}$ , and 38°C temperature. Three to five cultures under exponential growth phase were investigated under three stress conditions: high light, UV-B irradiance and microaerobiosis achieved with argon bubbling, followed by a recovery period with the standard conditions. Each culture was sampled three times during stress conditions, and two more times during the recovery period. The samples were used for total RNA extraction and for total protein extraction. We analyzed the expression pattern of the *psbA* and *hoxH* genes by real time PCR using specific primers, as well as the total pool of D1 protein by Western Blot.

The expression of the *psbA* genes turned out to be different under the three different types of stress. Under high light both the *psbA* genes encoding the D1 and the D1' proteins were induced, with some important differences of the expression during the recovery period. Same situation was observed under UV-B irradiance, with the higher gene expression being measured during the first minutes of treatment. The *hoxH* gene has not displayed a very high level of expression under microaerobiosis, as expected.

### P36: Protein components of a new transport complex in membranes of *Anabaena* sp. PCC 7120

**Carla Trigo<sup>1</sup>, Dinka Mandakovic<sup>1</sup> and Mónica Vasquez<sup>1</sup>**

<sup>1</sup>Laboratorio de Ecología Microbiana y Toxicología Ambiental, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile. Santiago, Chile.

Cyanobacteria have different morphologies, from unicellular to multicellular filamentous organisms. The filament development and maintenance depend on the exchange of materials and regulatory compounds among its structure. Important aspects of the development of the filament and how intercellular transfer of molecules occurs are still unclear [1]. *all1765*, a gene exclusive of filamentous cyanobacteria, encodes a hypothetical protein in *Anabaena* sp. PCC 7120 [2]. We found through *in silico* analyses that this protein has transmembrane segments and other domains with high similarity to proteins that belong to the type II secretion pathway (TIISP). Five genes with unknown function are arranged in the proximity of *all1765* forming a cluster that includes *all1765* and shows synteny in a wide number of filamentous cyanobacteria. Even more, two genes of this cluster, *all1769* y *all1770*, have similar characteristics to *all1765*, being also probable constituents of a TIISP. Considering these antecedents, we suggest that All1765, All1769 and All1770 form a protein complex in *Anabaena* sp. PCC 7120 membranes. The aim of this research is to identify the subcellular localization of these proteins and to determine their function. We generated a polyclonal antibody against All1765 and a GFP fusion to All1765. A membrane disposition of the protein in the filaments was observed. We have also generated a mutant strain for *all1765* in *Anabaena* sp. PCC 7120. The *all1765* mutant was evaluated in liquid medium with and without a nitrogen source (BG11 and BG11o respectively). A deficient growth and finally death of cultures was observed by standard light microscopy only in BG11o and in half of the colonies mutant obtained. We analyzed the ultra structure through transmission electron microscopy (TEM). The heterocyst of the mutant strain had normal envelope morphology and a probably normal fixation function, but showed a wider heterocyst neck compared to the wild type strain and a disarrangement of thylakoid membranes in vegetative cells. These results suggest a function of this protein in mechanisms involved in the diffusion of fixed nitrogen from heterocysts to vegetative cells. Further analysis must be done to definitely demonstrate this function. We are currently investigating the *all1769* and *all1720* genes with the same approaches described above for *all1765*.

[1] Mariscal, V., Flores, E. (2010) Multicellularity in a heterocyst-forming cyanobacterium: pathways for intercellular communication. *Adv Exp Med Biol.* 675:123-35;

[2] Stucken, K., John, U., Cembella, A., Murillo, A., Soto-Liebe, K., Fuentes, J., Friedel, M., Plominsky, A.M., Vásquez, M., Glockner, G. (2010) The smallest known genomes of multicellular and toxic cyanobacteria: comparison, minimal gene sets for linked traits and the evolutionary implications. *PLoS one* 5:e9235



### P37: Evidence for Multiple Carboxysomal Bicarbonate Dehydration Complexes based on the Form and Function of Carbonic Anhydrase

Charlotte de Araujo<sup>1</sup>, Matthew S. Kimber<sup>2</sup>, and George S. Espie<sup>1</sup>

<sup>1</sup>Dept. of Cell & Systems Biology, University of Toronto, Mississauga ON Canada; <sup>2</sup>Dept. of Molecular & Cellular Biology, University of Guelph, Guelph ON Canada

Carboxysomes are complex biochemical compartments essential for photosynthesis and the CO<sub>2</sub> concentrating mechanism in cyanobacteria. These prokaryotic organelles house Rubisco and the β-class carbonic anhydrase (CA), CcaA, within a protein shell. In *Synechocystis* PCC6803, the HCO<sub>3</sub><sup>-</sup> dehydration complex (BDC) is comprised of CcaA-CcmM-CcmN associated with the inner surface of the shell via CcmK. Functionally, CcmM acquires cytosolic HCO<sub>3</sub><sup>-</sup> and delivers it to CcaA which catalyzes HCO<sub>3</sub><sup>-</sup> dehydration within the carboxysome core [1]. However, not all cyanobacterial strains have a CcaA homolog.

We have identified two new variants of the BDC. The N-terminal region of the carboxysome protein CcmM has a γ-CA like domain. In *Synechocystis*, it is catalytically inactive, while CcmM from *Thermosynechococcus elongatus* BP-1 is catalytically active [2]. Purified recombinant CcmM from *T. elongatus*, *Gloeobacter violaceus* PCC7421 and *Nostoc* PCC7120 catalyzed <sup>18</sup>O exchange, a hallmark of all CAs. Using truncated forms of CcmM, biochemical analysis and homology modeling we defined the boundary of the γ-CA catalytic domain of CcmM to reside within the N-terminal 201 amino acids and identified cyanobacterial specific sequence elements that are required for γ-CA activity. CcmM181 and CcmM193 are inactive polypeptides, while CcmM196 displays 5.7% of the activity of CcmM209. CcmM201 is as active as the maximally active CcmM209, indicating an important stabilizing role for C194-C200. Consistent with this, is that the CcmM209 C194S, C200S mutant was 55% less active than the wildtype, though the Cys residues play no direct role in the catalytic mechanism. Reducing agents, DTT, TCEP and THP inhibited the γ-CA activity of *Nostoc* PCC7120 and *G. violaceus*, as did the physiological reductant glutathione. CA activity was reversibly restored by diamide, suggesting that the C194-C200 disulfide bond was critical for the oxidative activation and potential regulation of the enzyme. These data suggest that γ-CA activity of CcmM is the catalytic component of BDC in strains where genome analysis revealed the absence of a CcaA homologue. In other strains, such as *Cyanothece* ATCC51142 and *N. punctiforme* PCC73102, a hybrid form of the BDC exists as they possess a CcmM containing a γ-CA domain and a β-CA homolog that are both catalytically active. We suggest that the ancestral form of the BDC was the CcmM-CcmN complex as represented by *G. violaceus*. In other strains, CcaA was acquired through horizontal gene transfer leading to a CcaA-CcmM-CcmN complex with dual CA activity. A reduction in selection pressure within a subgroup of these strains may have led to a loss of CcmM CA activity, although CcmM continues to play a vital structural role within the BDC.

[1] Cot, S.S.W., et al., J. Bacteriol. 2008. 190(3): p. 936-945; [2] Peña, K.L., et al., PNAS 2010. 107(6): p. 2455-2460

### P38: Chemical Biology of 2-oxoglutarate signaling

Cheng-Cai Zhang<sup>1</sup>, Ling Peng<sup>2</sup>, Amel Latifi<sup>1</sup>, Zeinab Assaf<sup>2</sup>, Yang Wang<sup>2</sup>

<sup>1</sup>Laboratoire de Chimie Bactérienne – Aix-Marseille Université-CNRS, France; <sup>2</sup>Centre Interdisciplinaire de Nanoscience de Marseille, Aix-Marseille Université-CNRS, France

In cyanobacteria, the accumulation of 2-oxoglutarate (2-OG) within cells constitutes the signal of nitrogen starvation and elicits cell responses accordingly, such as heterocyst differentiation in *Anabaena* PCC 7120. One receptor of 2-OG is NtcA, a transcription factor. The binding of 2-OG on NtcA stabilizes the NtcA dimer and optimizes the distance between the two F-helices for DNA binding (1, 2). We have synthesized and characterized a series of 2-OG analogues and these studies allowed us to get important insight into the structure-function relationship of 2-OG (3-4). Similarly, we are developing a system that would allow us to identify potential new receptors of 2-OG. For that purpose, we have modified different positions of 2-OG and analyzed the effect of such modifications on the 2-OG signaling function. Finally, a series of affinity resins have been prepared which attached 2-OG to a linker that can differ in size and charge. These affinity columns are being tested with different cellular extracts, and the preliminary results will be reported.

[1] Laurent, S., Laurent, S., Chen, H., Bédou, S., Ziarelli, F., Peng, L. & Zhang, C.-C. (2005) Nonmetabolizable analogue of 2-oxoglutarate elicits heterocyst differentiation under repressive conditions in *Anabaena* sp. strain PCC 7120. Proc. Natl. Acad. Sci. USA. 102:9907-9912; [2] Zhao, MX, Zhao, M.-X., Jiang, Y.-L., He, Y.-X., Chen, Y.-F., Teng, Y.-B., Chen, Y., Zhang, C.-C., and Zhou, C.-Z. (2010) Structural basis for the allosteric control of the global nitrogen-regulating transcription factor NtcA by the nitrogen starvation signal 2-oxoglutarate. Proc. Natl. Acad. Sci. USA. 107 :12487-12492; [3] Chen, H., Laurent, S., Bédou, S., Ziarelli, F., Cheng, Y., Zhang, C.-C. & Peng, L. (2006) Studying the signaling role of 2-oxoglutaric acid by using analogues that mimic the ketone and ketal forms of 2-oxoglutaric acid. Chemistry & Biology. 13:849-856; [4] Liu, X., Chen, H., Laurini, E., Wang, Y., Dal Col, V., Posocco, P., Ziarelli, F., Fermeglia, M., Zhang, C.-C., Priel, S., and Peng, L. (2011) 2-Difluoromethylene-4-methylenepentanoic Acid, A Paradoxical Probe Able To Mimic the Signaling Role of 2-Oxoglutaric Acid in Cyanobacteria. Org. Lett. 13 :2924-2927

### P39: OCP related photoprotective mechanism in phycobilisome core mutants of the cyanobacterium *Synechocystis* PCC 6803

Denis Jallet<sup>1,2</sup>, Michal Gwizdala<sup>1,2</sup> and Diana Kirilovsky<sup>1,2</sup>

<sup>1</sup>CEA, iBiTec-S, Gif sur Yvette, France; <sup>2</sup>CNRS, UMR 8221, 91191 Gif sur Yvette, France

Cyanobacteria have developed a unique photo-protective mechanism to decrease Reactive Oxygen Species formation at the level of reaction centers under saturating light conditions. It relies on the Orange Carotenoid Protein (OCP) that gets converted from an orange inactive form (OCP<sup>0</sup>) to a red active one (OCP<sup>R</sup>) when submitted to a strong blue-green illumination. It then binds to the major light harvesting antenna – the phycobilisomes (PBs) – and induces dissipation of the energy they collect as heat. Concomitantly, a decrease in (quenching of) fluorescence is observed. Using an *in vitro* reconstitution system our team showed that a single OCP<sup>R</sup> is bound to each quenched PB at the level of the core. PB cores contain bulk allophycocyanin trimers (ApcA, ApcB emission at 660 nm) and trimers containing red-shifted terminal emitters (ApcD, ApcE, ApcF emission at 680 nm). Each trimer is a possible site for OCP binding. *Synechocystis* PCC 6803 mutant strains affected in their terminal emitters were constructed. Deletion strains were obtained for *apcD* and *apcF* genes using antibiotic resistance cassettes. Because it is impossible to suppress ApcE (i.e Lcm; its suppression leads to disassembled cores), its chromophore-binding cysteine was replaced by a serine in the *apcEC190S* point mutant. In all the strains, high intensities of blue-green light triggered fluorescence quenching indicating the presence of the photo-protective mechanism. Amplitude and kinetics of fluorescence quenching were similar in mutants and wild-type. For a further characterization, phycobilisomes from all the mutants were purified and the quenching tested *in-vitro*. Once again, no differences were observed. None of the terminal emitters is absolutely required for quenching induction. Our results strongly suggest that OCP interacts with bulk allophycocyanin emitting at 660nm in the phycobilisome core.

## P40: Understanding prokaryotic multicellularity – Intercellular communication in the true-branching cyanobacterium *Mastigocladus laminosus*

**Dennis J Nürnberg<sup>1</sup>, Jamie Parker<sup>1</sup>, Giulia Mastroianni<sup>1</sup>, Enrique Flores<sup>2</sup>, and Conrad W Mullineaux<sup>1</sup>**

<sup>1</sup>School of Biological and Chemical Sciences, Queen Mary, University of London, London, UK; <sup>2</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, Seville, Spain

The filamentous, heterocyst-forming cyanobacterium *Mastigocladus laminosus* is one of the morphologically most complex prokaryotes. It is characterised by cellular division in more than one plane, resulting in the formation of true branches, and by a high degree of cell differentiation.

Ultrastructural investigations by transmission electron microscopy revealed structures that are likely involved in intercellular communication. A gene coding for the potential key protein in intercellular communication, SepJ, was present. It consists of the full three domains (coiled-coil, linker and permease domains), found in SepJ of all other filamentous, heterocyst-forming cyanobacteria.

Under nitrogen deprivation, almost any cell is capable of differentiating into a heterocyst. A heterocyst at the branching point forms three cyanophycin plugs to the adjacent three vegetative cells, supporting the idea of connectivity of branch and main filament and pointing to a possible role of wide filaments as distributors of fixed nitrogen to the rapidly growing branches. Trichome breakage by the formation of necridia is as random as heterocyst formation. Several necridia can be found within a filament at almost any position, inhibiting further molecular exchange between neighbouring cells.

To directly investigate intercellular communication in *M. laminosus*, we loaded the fluorescent tracer 5-carboxyfluorescein diacetate into cells, and determined its transfer by fluorescence recovery after photo bleaching.

Our results demonstrate the connectivity of branch and main filaments, enabling the exchange of molecules throughout the entire filament network. We observed that exchange between cells within a culture is regulated, depending on their stage in the life cycle. Young, narrow filaments and hormogonia (motile filaments) exhibited rapid exchange rates among cells, whilst old, wide filaments showed reduced rates, although they remained connected.

## P41: Molecular and morphological characterization of a new cyanobacterial species *Pseudanabaena arctica*

**Otakar Strunecký<sup>1,2</sup>, Jiří Komárek<sup>1</sup> and Josef Elster<sup>1,2</sup>**

<sup>1</sup>Institute of Botany, Centre for Phycology, The Academy of Sciences of the Czech Republic; <sup>2</sup>Centre for Polar Ecology, Faculty of Science, University of South Bohemia, Czech Republic

*Pseudanabaena* species are poorly known filamentous cosmopolitan cyanobacteria (Acinas, *et al.*, 2008). The family Pseudanabaenaceae is well defined by phenotype as well as molecular characters. They are traditionally classified as closely related to *Limnothrix*, *Pseudanabaena*, *Romeria* and *Planktolyngbya* (Komárek & Anagnostidis, 2005, Komárek, 2008). We examined a strain CCALA 873 collected at Hornsund, Svalbard, which was morphologically consistent with *Pseudanabaena*. The 16S rDNA of the strain CCALA 873 was extracted and sequenced. The strain was also examined for thylakoid structure and cell division type with TEM as well as traditional morphology with LM. Constructed phylogenetic trees using parsimony, distance, and maximum likelihood methods were similar in topology, represented an isolated cluster according to 16S rRNA gene sequencing. Because the whole group of strains genetically similar to CCALA 873 obtained by BLAST from Genbank represented a phylogenetically distinctly separated cluster, we described it as a special taxon *Pseudanabaena arctica* in agreement with Botanical Nomenclatoric Code (ICBN, 2006). The genetically most related clusters always have genetic similarity less than 95% and differ by distinct autapomorphic features.

[1] Acinas SG, Haverkamp THA, Huisman J & Stal LJ (2008) Phenotypic and genetic diversification of *Pseudanabaena* spp. (cyanobacteria). ISME J 3: 31-46; [2] ICBN (2006) International Code of Botanical Nomenclature (Vienna Code). (McNeill J., Burdet H.M., Demoulin V., Hawksworth D.L., Marhold K., Nicolson D.H., Prado J., Silva P.C., Skog J.E., Wiersema J.H. & Turland N.J., eds.), Gantner Verlag, Vienna, Austria; [3] Komárek J (2008) Recent changes in cyanobacterial taxonomy based on combined molecular, phenotype and ecological consequences; [4] Komárek J & Anagnostidis K (2005) Cyanoprokaryota -2. Teil/ 2nd Part: Oscillatoriales. Elsevier/Spektrum, Heidelberg.

## P42: Identification of a novel polysaccharide secretion system essential for gliding motility in *Nostoc punctiforme*

**Douglas D. Risser and John C. Meeks**

Department of Microbiology, University of California, Davis, California 95616

Many cyanobacteria are capable of gliding motility, the ability to move smoothly across solid surfaces. This motility has only been well defined in the unicellular cyanobacterium *Synechocystis* sp. strain PCC6801, which utilizes twitching motility via type IV pili to glide in a social manner at rates of about 2  $\mu\text{m}/\text{min}$ . In contrast, gliding motility in filamentous cyanobacteria remains poorly defined, but the faster rate of motility, about 2  $\mu\text{m}/\text{s}$  and the lack of a social component imply a different mechanism of gliding in these strains. Some genera, such as *Nostoc* spp. are motile only as hormogonia, short chains of morphologically distinct cells. It has been postulated that gliding motility is driven by the secretion of polysaccharides, colloquially referred to as the "slime gun" model of gliding motility, and electron microscopy has identified a ring of putative polysaccharide secretion pores, termed junctional pores, adjacent to the cell septum in numerous gliding filamentous cyanobacteria. However, to date the identity of proteins comprising these pores remains unknown and their role in motility remains undefined. We recently characterized a chemotaxis-like gene cluster, designated *che2*, in *Nostoc punctiforme* and found it to be essential for both motility and polysaccharide secretion. Intriguingly, proteins from this cluster localized to a ring at each end of the cell adjacent to the septum, placing it in close proximity to the putative site of the junctional pores. Comparative transcriptomics with a mutant from this locus led us to investigate a previously uncharacterized gene cluster. This locus contains a number of glycosyl-transferases, hallmarks of polysaccharide synthesis, which are upregulated in wild-type hormogonia but not in a *che2* mutant strain. Deletions in three different regions of this cluster resulted in non-motile filaments that no longer secrete polysaccharide, supporting the hypothesis that this cluster encodes a polysaccharide secretion system essential for gliding motility. Several components of this cluster appear to be unique to filamentous cyanobacteria implying a common mechanism for gliding in these organisms. We speculate that this locus may encode for components of the junctional pores, the putative motor driving gliding motility in filamentous cyanobacteria. Alternatively, while essential for motility, polysaccharide secretion may play a more accessory role, such as providing an appropriate matrix for gliding via another mechanism.

## P43: The influence of light on carbon utilization in Aerobic Anoxygenic Phototroph

**Dzmitry Hauruseu<sup>1,2</sup> and Michal Koblížek<sup>1,2</sup>**

<sup>1</sup>Institute of Microbiology CAS, Opatovický mlýn, 379 81 Třeboň, Czech Republic; <sup>2</sup>Faculty of Science, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic

Aerobic anoxygenic phototrophs are prokaryotes which harvest light energy using bacteriochlorophyll-containing reaction centres. In this study we investigated the efficiency of their carbon metabolism in carbon-limited chemostat cultures. *Erythrobacter* sp. NAP1 and *Roseobacter* sp. COL2P were grown in carbon limited chemostat regime on defined carbon sources (glutamate, pyruvate, acetate, fumarate, leucine, glucose) and illuminated with different light intensities. When grown in a light-dark cycle these bacteria accumulated 25 - 110% more biomass in terms of carbon when compared to the cultures grown in the dark. Cultures grown on glutamate accumulated most of the biomass at 150  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , but were inhibited at higher light intensities. In the case of pyruvate we did not find any inhibition of growth by high irradiance. In addition, the incorporation of radiolabeled bicarbonate was studied in cultures of *Erythrobacter* sp. NAP1. In the culture grown on pyruvate there was a strong stimulation of carbon incorporation by light. In contrast, glutamate grown cultures showed almost no effect of light bicarbonate incorporation. The enhanced efficiency of aerobic anoxygenic phototrophs in carbon utilization might be an important competitive advantage when growing under carbon limited conditions.

## P44: Variability of storage $\alpha$ -glucans (glycogen or starch) among unicellular, nitrogen-fixing cyanobacteria

**Eiji Suzuki<sup>1</sup>, Jun Fukushima<sup>2</sup>, Kojiro Hara<sup>1</sup>, Yoichiro Shimura<sup>2</sup>, Tomokazu Konishi<sup>3</sup>, Ryuichiro Suzuk<sup>1</sup>, Keiju Okano<sup>2</sup>, Tamio Inamoto<sup>2</sup>, Yasunori Nakamura<sup>1,4</sup>**

<sup>1</sup>Department of Biological Production, <sup>2</sup>Department of Biotechnology and <sup>3</sup>Department of Biological Environment, Faculty of Bioresource Sciences and <sup>4</sup>Board of Directors, Akita Prefectural University, Akita, Japan

Cyanobacteria synthesize and store  $\alpha$ -glucan as one of the photosynthetic products. We have shown that the storage of  $\alpha$ -glucan plays a pivotal role for these organisms to cope with the hostile environment (ex. high-salt and oxidative stresses)[1]. While most cyanobacterial species produce soluble glycogen as the storage  $\alpha$ -glucan, some unicellular, nitrogen-fixing species (or species that have the nitrogenase gene) accumulate water-insoluble polysaccharide, which is highly analogous to amylopectin found in plants[2]. A few species also contain amylose as a minor component in addition to amylopectin-like  $\alpha$ -glucan[3]. These insoluble polysaccharides exhibit physicochemical characteristics such as granular morphology, crystallinity and thermal pasting property commonly observed for starch. These  $\alpha$ -glucans can be therefore referred to as "cyanobacterial starch". The water-insoluble, osmotically inert polysaccharide would be advantageous for accumulation in large amounts to ensure high rates of respiration and production of much energy during the night for the strictly anaerobic process of nitrogen fixation. It is interesting to test whether the accumulation of starch-like polysaccharide is the prerequisite for the nitrogen-fixing capability. In the present study, the form of storage  $\alpha$ -glucan was investigated in *Cyanothece* strains. Glycogen and amylopectin can be explicitly distinguished by chain length distribution analysis using capillary electrophoresis after enzymatic debranching treatment at  $\alpha$ -1,6-linkage and fluorescent labeling at the reducing end. The most abundant chains are found at degree of polymerization (DP) 6 to 8 for glycogen and DP 11 to 12 for amylopectin. The polysaccharide species in *Cyanothece* sp. ATCC 51142, PCC 8801 and PCC 8802 was amylopectin, while that in *Cyanothece* strains PCC 7424 and PCC 7425 was glycogen. The number of genes responsible for the metabolic steps of  $\alpha$ -glucan biosynthesis is variable based on the available genomic sequences of these organisms. A possible mechanism underlying the production of distinct  $\alpha$ -glucan is discussed.

[1] Suzuki E, Ohkawa H, Moriya K, Matsubara T, Nagaike Y, Iwasaki I, Fujiwara S, Tsuzuki M, Nakamura Y (2010) Appl Environ Microbiol 76: 3153-3159; [2] Nakamura Y, Takahashi J-I, Sakurai A, Inaba Y, Suzuki E, Nihei S, Fujiwara S, Tsuzuki M, Miyashita H, Ikemoto H, Kawachi M, Sekiguchi H, Kurano N (2005) Plant Cell Physiol 46: 539-545 [3] Deschamps P, Colleoni C, Nakamura Y, Suzuki E, Putaux J-L, Buléon A, Haebel S, Ritte G, Steup M, Falcón LI, Moreira D, Löffelhardt W, Raj JN, Plancke C, d'Hulst C, Dauvillée D, Ball S. (2008) Mol Biol Evol 25: 536-548

## P45: The sRNA repeat RSs0680a-d modulates C1-metabolism in *Rhodobacter sphaeroides* under specific stress conditions

Fabian Billenkamp<sup>1</sup>, Bork Berghoff<sup>1</sup> and Gabriele Klug<sup>1</sup>

<sup>1</sup>Institute for Molecular and Microbiology, Justus-Liebig-University Giessen, Giessen, Germany

When dealing with environmental changes bacteria employ a network of regulatory factors that are based mainly on proteins but also on regulatory RNAs. Most stress responses are triggered by such regulatory networks. A special type of such a regulatory response is the post transcriptional gene regulation by small RNAs (sRNAs). Although there are few sRNAs that bind proteins, common sRNAs bind to target messenger RNAs (mRNAs) and modulate the stability and/or translation of the mRNA [1]. There are two classes of sRNAs interacting with mRNAs, the so called cis-encoded antisense RNAs that show perfect base pairing with their target mRNAs and the trans-encoded sRNAs which show limited complementarity to their target mRNAs. To facilitate the interaction, the hexameric Sm-like protein Hfq is needed in case of trans-encoded sRNAs to overcome limited base pairing [1]. In *Rhodobacter sphaeroides* the expression of several such sRNAs is related to (photo-) oxidative stress [2]. One trans encoded sRNA that shows increased expression levels under (photo-) oxidative stress is RSs0680a, which is cotranscribed with 3 homologous sRNAs (RSs0680b-d) and one hypothetical protein (RSP\_6037). To proceed stress dependent induction, the RSP\_6037/RSs0680a-d operon is controlled by an RpoH<sub>I</sub>/RpoH<sub>II</sub>-dependent promoter, which is induced under (photo-)oxidative stress and heat stress[3]. We could show that overexpression of RSs0680a-d in *R. sphaeroides* leads to enhanced resistance to oxidative stress. A transcriptome analysis revealed several mRNAs with changed abundance in the *R. sphaeroides* RSs0680a-d overexpression strain. Many of these mRNAs show relation to the glutathione (GSH) dependent formaldehyde metabolism. A combination of this transcriptome analysis with different bioinformatic approaches pointed out, that the mRNA of *flhR* (RSP\_2591), a transcriptional activator in the GSH dependent formaldehyde metabolism, is a putative target of RSs0680a-d. Interaction of RSs0680a with the *flhR* mRNA could be verified in vitro by electro mobility shift assays and *in vivo* by use of a reporter system for mRNA-sRNA interactions. Moreover we observed increased GSH levels in the RSs0680a-d overexpression strain in comparison to an empty vector control. This leads us to the conclusion that RSs0680a-d are helping to increase the free GSH pool under stress conditions by repressing a metabolic pathway that uses high amounts of GSH.

[1] Waters, L.S., Storz, G. (2009) Regulatory RNAs in Bacteria. Cell 136: 615-628; [2] Berghoff B.A., Glaeser J., Sharma C.M., Vogel J., Klug G. (2009) Photooxidative stress induced and abundant small RNAs in *Rhodobacter sphaeroides*. Mol Microbiol 74: 1497-512; [3] Nuss A.M., Glaeser J., Berghoff B.A., Klug G. (2010) Overlapping Alternative Sigma Factor Regulons in the Response to Singlet Oxygen in *Rhodobacter sphaeroides*. J Bacteriol 192: 2613-2623.

## P46: Effect of exudates from toxic strains of *Microcystis aeruginosa* (Cyanobacteria) on their development

Fernanda Rios Jacinavicius<sup>1,2</sup>, Filipe Brigatto Mariano<sup>1</sup>, Célia Leite Sant'Anna<sup>1</sup> and Luciana Retz de Carvalho<sup>1</sup>

<sup>1</sup>IBT- Nucleus of Phycology, Institute of Botany , 04301-902, São Paulo, Brazil; <sup>2</sup>PhD Student, Institute of Botany, Brazil, grant support of FAPESP (Process 2011/50267-8) rios\_bio@yahoo.com.br

Some hypotheses indicate that microcystins present allelopathic activity. However, there is not yet a clear understanding of their function and control production. Aiming to learn about the effects of the exudate of *Microcystis aeruginosa* strains on their growth and morphology, we used two strains: one produces greater concentration of microcystin RR and less LR and YR (CCIBt3194) and the other produces microcystin YR as the dominant toxin and a small amount of RR and LR (CCIBt3454). The strains were isolated from the same drinking water supply in Brazil, at different time periods: August 2001(CCIBt3194) and January 2011 (CCIBt3454). Both strains are kept in the Cyanobacteria Culture Collection of the Institute of Botany, Brazil, under controlled conditions: irradiance 40-50  $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ , temperature 23+2oC, medium ASM-1 (pH 7.4) and photoperiod 14:10h light-dark cycle. These conditions were considered as control (n=3). Treatment 1: CCIBt3194 strain grew in a medium previously conditioned by the exudate of CCIBt3454 for 15 days. Treatment 2: the same as Treatment 1, but the study was performed with CCIBt3454 strain growing in the exudate of CCIBt3194. The initial number of cells was identical for controls and treatments ( $1,5 \times 10^5$  cells.mL<sup>-1</sup>). For 17 days, daily growth (cells.mL<sup>-1</sup>) and morphometric (n=30) analyses were carried out for the treatments and controls. The applied statistical analyses were ANOVA and Tukey Test. The highest growth rates ( $\mu$ ) and cell yield (R; cells.mL<sup>-1</sup>) were observed in the controls. In the treatments, CCIBt3454-MCYR presented  $\mu=0,148$  while CCIBt3194-MCRR did not present growth rate. CCIBt3194-MCRR was intensely affected in Treatment 1, having a 64% cell yield reduction when compared with the control. On the other hand, CCIBt3454-MCYR was much less affected in Treatment 2, having only a 23% decrease in cell yield. As for morphology, there is strong evidence that microcystins influence cell volume and are mainly produced by large colonies. CCIBt3194-MCRR formed large aggregates and increased cell diameter when compared with the control. CCIBt3454-MCYR strain presented the opposite result in contact with the exudate, forming more disperse colonies and increasing cell diameter only on the first and second cultivation days. This strain started growing after the fifth cultivation day when it presented a rusty color. These results indicate that microcystins produced by the same specie induced different responses according to the dominant type of the variable. Therefore, there is indication that the exudate where microcystin-YR was predominant caused more effect on the development when compared with the exudate where microcystin-RR was predominant, supporting the hypothesis that microcystins and/or other compounds can inhibit the growth of other organisms, including *Microcystis aeruginosa*.



**P47: Diversity and evolution of chromatic adaptation in marine *Synechococcus* cyanobacteria****Florian Humily<sup>1</sup>, Christophe Six<sup>1</sup>, Laurence Garczarek<sup>1</sup>, Animesh Shukla<sup>2</sup>, David Kehoe<sup>2</sup>, Dominique Marie<sup>1</sup> and Frédéric Partensky<sup>1</sup>**<sup>1</sup>UPMC-Université Paris VI and CNRS, Station Biologique, Oceanic Plankton Group, Place Georges Teissier, 29680 Roscoff, France; <sup>2</sup>Department of Biology, 1001 East Third Street, Indiana University, Bloomington, IN47405, USA

Chromatic adaptation (CA) provides a variety of cyanobacteria with the ability to match the properties of their light-harvesting complex, called the phycobilisome (PBS), to the quality (or color) of ambient light [1]. CA has been most studied in freshwater cyanobacteria and consists in changes in the relative contents of phycoerythrin (PE) and/or phycocyanin, when cells are shifted from red to green light or vice versa. In contrast, in marine *Synechococcus*, another process, called Type IV CA (hereafter CA4), occurs during shifts from blue (BL) to green light (GL) or vice versa [2]. CA4 does not involve changes in the relative contents of phycobiliproteins, like it does in freshwater cyanobacteria, but in the chromophorylation of some PE subunits [3]. Indeed, the ratio of the two bilins bound to both PEI and PEII, phycoerythrin (PEB;  $A_{max}=495$  nm) and phycoerythrobilin (PEB;  $A_{max}=550$  nm) is high under blue light and low under green or white light [2, 3]. Recently, an enzyme responsible for this process, MpeZ, which binds PEB at Cys-83 of alpha-PEII (MpeA) and isomerizes it into PUB has been characterized in strain RS9916 [4]. CA4 is not restricted to a single clade of *Synechococcus* but seems to be widely distributed among this genus. In order to better characterize the variability of this process, we compared the physiological responses of 13 potentially chromatically adapting isolates. Strains acclimated to BL and GL were respectively shifted to GL and BL and this experiment was done at two distinct irradiances (20 and 80  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ). This study showed that the kinetics of acclimation and the amplitude of variation of the PUB to PEB ratio may vary between strains, independently from their phylogenetic position in the *Synechococcus* radiation. We also found that the kinetics of the CA4 process was correlated to the growth rate. Finally we showed that the CA4 process requires protein synthesis. We hypothesize that CA4 implies biosynthesis of novel PEII discs, with a pigmentation matching ambient light quality and that PBS with the novel chromophorylation progressively replace initial PBS. This study provides new insights into understanding the ecological success of *Synechococcus* in variable marine environments, such as coast-offshore transition zones.

[1] Gutu, A., and Kehoe, D.M. (2011) Emerging Perspectives on the Mechanisms, Regulation, and Distribution of Light Color Acclimation in Cyanobacteria. *Mol. Plant* doi: 10.1093/mp/ssr054: 1-13; [2] Palenik, B. (2001) Chromatic adaptation in marine *Synechococcus* strains. *Appl. Environ. Microbiol.* 67 (2): 991-994; [3] Everroad, C., Six, C., Partensky, F., Thomas, J.C., Holtzendorff, J., and Wood, A.M. (2006) Biochemical bases of type IV chromatic adaptation in marine *Synechococcus* spp. *J. Bacteriol.* 188 (9): 3345-3356; [4] Shukla, A., Schlachter W.M., Blot N., Partensky F., L. Hammad L., Garczarek, Gutu a., J. A. Karty and D. Kehoe. Characterization of a key enzyme involved in Type IV chromatic adaptation in marine *Synechococcus* cyanobacteria. Submitted to *Proc Natl Acad. Sci. U.S.A.*

**P48: Glucose-sensitivity of *Synechocystis* sp. PCC 6803 under the light is due to the lack of regulatory ability of PSI and thylakoids****Fumihiko Itoh<sup>1</sup>, Masahiro Karita<sup>1</sup>, Yoshihiro Shiraiwa<sup>2</sup>, Iwane Suzuki<sup>2</sup>**<sup>1</sup>Graduate School of life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan; <sup>2</sup>Faculty of Life and Environmental Sciences, University of Tsukuba, Ibaraki, Japan

In the cyanobacterium *Synechocystis* sp. PCC 6803 there are two types of wild-type strains, one is the glucose-tolerant (GT) strain represented by William's or Kazusa strains and another is the glucose-sensitive (GS) strain represented by PCC 6803 or ATCC 27184 strains[1]. Although there are several variations in the genomic sequences in the GS or GT strains, it is not clear how the genetic variations are really involved in the different responses to glucose.

Growth of both GT (Kazusa strain) and GS cells (PCC strain) were continued for 12 h after addition of 10 mM glucose in the BG-11 medium, but the growth of GS cells was halted in 24 h. The activities of photosynthesis and respiration in the GS cells were gradually increased for 12 h after addition of glucose. However, those in the GT cells are shortly increased after the addition of glucose and then reduced for further longer incubation, i.e. for 12 h. The DNA microarray analyses showed that repression in expression of the genes for PSI subunits was lesser extent in the GS cells than the GT cells, suggesting that the GS cells lacked the regulation of electron transfer on the thylakoid membranes after the addition of glucose. Analyses of the chlorophyll-fluorescence emission spectra at 77 K in the cells grown under the photomixotrophic conditions showed that the PSII/PSI ratio was decreased in the GS strain under the conditions, suggesting that PSI activity in the GS cells was remained high level under the conditions

We observed changes in cell morphologies in both cells by transmission electron microscope after the addition of glucose. It showed that the thylakoid membranes were decreased in the GT cells cultured for 12 h after the addition of glucose, corresponding to reduction of photosynthetic activity. However, in the GS cells the thylakoid membranes were fragmented and formed plenty of small vesicles with low electron density. Administration of a glucose analogue, 3-O-methyl glucose, did not induce any effects on both strains, indicating that metabolism of glucose might induce the effects.

These results suggest the GS cells lack the regulatory mechanisms for respiration/photosynthesis and PSII/PSI ratios under the presence of glucose while GT cells maintain the activities.

[1] Ikeuchi et al. (2001) *Photosynthesis Res.* 70, 73-83

## P49: Cyanobacteriochrome Tlr1999 is a teal-light-activated c-di-GMP phosphodiesterase.

**Gen Enomoto<sup>1</sup>, Rei Narikawa<sup>1,2</sup>, and Masahiko Ikeuchi<sup>1,3</sup>**

<sup>1</sup>Department of Life Sciences (Biology), Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan; <sup>2</sup>Japan Science and Technology Agency (JST), PRESTO, Saitama, Japan; <sup>3</sup>Japan Science and Technology Agency (JST), CREST, Saitama, Japan

Cyanobacteriochromes (CBCRs) are cyanobacterial photoreceptors that regulate phototaxis, complementary chromatic acclimation, etc. [1]. CBCRs are related to phytochromes. Both photoreceptors utilize a linear tetrapyrrole (bilin) chromophore that is covalently anchored at a conserved cysteine residue for their reversible photoconversion. CBCRs, however, have diverse spectral and photochemical properties throughout the near-UV/visible region and a wide variety of domain compositions. These features contrast with those of phytochromes, which respond to red/far-red light and have well-conserved domain organizations. Thus it is crucial to elucidate the molecular mechanisms of CBCRs' unique photoconversion and light-activation/inactivation of their output domains.

Recently, we analyzed the chromophore-binding GAF domain of a CBCR Tlr1999 from *Thermosynechococcus elongatus* and showed that exogenous free thiols can mimic the second conserved cysteine, which reversibly binds the bilin chromophore during its blue/teal-type photoconversion [2]. Here, we studied the full-length of Tlr1999. His-tagged full-length Tlr1999 protein was prepared from *E. coli* and cyanobacterium *Synechocystis*. The full-length protein showed blue/teal photoconversion that was indistinguishable from the sole GAF domain. Tlr1999 comprises GAF, GGDEF, and EAL domains. In general, GGDEF or EAL domain serves as a diguanylate cyclase or a phosphodiesterase for bacterial second messenger bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP), respectively. We measured the activity of c-di-GMP synthesis and degradation for the full-length Tlr1999 using HPLC-based assays. The results suggested that Tlr1999 does not work as a diguanylate cyclase but a phosphodiesterase. The phosphodiesterase activity was activated by teal-light, GTP, high temperature, and high pH. Previous study suggested that a blue/green-type CBCR Tlr0924 causes cell aggregation under blue light via its diguanylate cyclase activity. Tlr1999 may work in a coordinated manner with Tlr0924 to enable fine regulation of cell aggregation and other responses, depending on the ambient light quality especially in blue-to-teal/green ratio.

[1] Ikeuchi, M. and T. Ishizuka (2008). Photochem. Photobiol. Sci. 7(10): 1159-67; [2] Enomoto, G. et al. (2012). Biochemistry 51(14): 3050-3058.

## P50: Rising CO<sub>2</sub> and Harmful Cyanobacteria

**Giovanni Sandrini, Hans C. P. Matthijs and Jef Huisman**

Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

Climate change scenarios predict a doubling of the atmospheric CO<sub>2</sub> concentration by the end of this century. Phytoplankton, including cyanobacteria, is responsible for ~50% of the global CO<sub>2</sub> fixation. Yet, how rising CO<sub>2</sub> will affect the species composition of aquatic microbial communities is still largely an open question. Several cyanobacterial species, like *Microcystis*, can produce harmful toxins, including microcystins. Competition studies showed that at low CO<sub>2</sub>/high light conditions a toxic *Microcystis aeruginosa* strain dominated over a non-toxic homologue, while the non-toxic strain dominated at elevated CO<sub>2</sub> levels/low light conditions (Van de Waal *et al.* 2011). This connects well to the suggested protein-modulating function of microcystin, protecting RuBisCO and other proteins against oxidative stress (Zilliges *et al.* 2011). The question we pose is why different CO<sub>2</sub> concentrations give rise to strain specific responses: is this related to the presence of microcystin, or are factors involved? A variety of *M. aeruginosa* strains has been grown under different CO<sub>2</sub>, pH and salt conditions. Data will be presented that investigate relationships between inorganic carbon uptake, microcystin production, oxidative stress tolerance and strain competitiveness.

[1] Van de Waal, D. B., *et al.* (2011) Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO<sub>2</sub>. ISME J 5:1438-1450; [2] Zilliges, Z., *et al.* (2011) The cyanobacterial hepatotoxin microcystin binds to proteins and increases the fitness of *Microcystis* under oxidative stress conditions. PLoS One 6:e17615.

\* This work was supported by a TOP project grant from the Netherlands Organisation for Scientific Research.

## P51: High-level production of the industrial product, lycopene, using the photosynthetic bacterium, *Rhodospirillum rubrum*

**Guo-Shu Wang<sup>1</sup>, Hartmut Grammel<sup>2</sup>, Khaled Abou-Aisha<sup>1</sup>, Rudolf Sägeser<sup>1</sup>, and Robin Ghosh<sup>1\*</sup>**

<sup>1</sup>Department of Bioenergetics, Institute of Biology, University of Stuttgart, Stuttgart, Germany, D-70569 Stuttgart, Germany; <sup>2</sup>Max-Planck-Institute for the Dynamics of Complex Technical Systems, Sandtorstr.1, D-39016 Magdeburg, Germany

The biosynthesis of the major carotenoid, spirilloxanthin, of the purple non-sulphur bacterium, *Rhodospirillum rubrum*, is thought to occur via a linear pathway proceeding through phytoene and later lycopene as intermediates. This assumption is based solely on early chemical evidence (Davies, B. H. 1970. Biochem. J. 116:93-99). In most purple bacteria, the desaturation of phytoene, catalyzed by the enzyme phytoene desaturase (CrtI) leads to neurosporene, involving only three dehydrogenation steps, and not four as in the case of lycopene. Here we show that the chromosomal insertion of a kanamycin resistance cassette into the *crtC-crtD* region of the partial carotenoid gene cluster, whose gene products are responsible for the downstream processing of lycopene, leads to the accumulation of the latter as the major carotenoid. We provide spectroscopic and biochemical evidence that *in vivo*, lycopene is incorporated into the light-harvesting complex 1 as efficiently as the methoxylated carotenoids spirilloxanthin (in the wild-type) and 3,4,3',4'-tetrahydrospirilloxanthin (in a *crtD*-mutant), both under semi-aerobic, chemoheterotrophic, as well as photosynthetic, anaerobic conditions. Quantitative growth experiments under dark, semi-aerobic conditions, using a growth medium for high cell density/high intracellular membrane levels, suitable for conventional industrial production in the absence of light, yielded values for lycopene of up to 2 mg/g cellular dry weight, or up to 15 mg/liter culture. These values are comparable to those of many previously described *Escherichia coli* strains engineered for lycopene production. The study provides the first genetic proof that the *R. rubrum* CrtI produces lycopene exclusively as an end product.

[1] Davies, B. H. 1970. A novel sequence for phytoene dehydrogenation in *Rhodospirillum rubrum*. Biochem. J. 116:93-99.



## P52: Formation of prolamellar body-like ultrastructures in the cyanobacterium *Leptolyngbya boryana* by co-accumulation of light-dependent protochlorophyllide reductase and protochlorophyllide in the dark

Haruki Yamamoto<sup>1</sup>, Hiroko Kojima<sup>1</sup>, Kaori Ohki<sup>2</sup>, and Yuichi Fujita<sup>1</sup>

<sup>1</sup>Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; <sup>2</sup>Department of Marine Bioscience, Faculty of Biotechnology, Fukui Prefectural University, Fukui, Japan

Chlorophyll *a* (Chl), a tetrapyrrole pigment is essential for photosynthesis and synthesized from glutamate via a complex pathway. At the penultimate step of Chl *a* biosynthesis, there are two different enzymes for protochlorophyllide (Pchl) reduction; one is dark-operative Pchl oxidoreductase (DPOR) and the other is light-dependent Pchl oxidoreductase (LPOR) [1]. Since angiosperms have only LPOR, seedlings of angiosperm are etiolated in the dark. In contrast, most oxygenic photosynthetic organisms such as gymnosperms, algae and cyanobacteria have the ability to synthesize Chl even in the dark due to the presence of DPOR. Dark-grown angiosperm seedlings accumulate a large amount of Pchl that exists as a photoactive ternary complex, Pchl-LPOR-NADPH, in the etioplasts to form prolamellar body (PLB) characterized by a unique paracrystalline structure. Upon illumination, Pchl is converted to Chl in PLB triggering the development of thylakoid membranes. Considering the wide distribution of LPOR from cyanobacteria to angiosperms, it would be interesting to understand whether cyanobacterial LPOR has the ability to form PLB. In this work, the *por* gene encoding LPOR from the cyanobacterium *Leptolyngbya boryana* was overexpressed in a mutant YFC2 lacking DPOR of *L. boryana*, and the YFC2 cells with the LPOR-overexpression plasmid (YFC2/P202) were observed by electron microscopy. YFC2 accumulates Pchl in the dark like angiosperm seedlings to give rise to 'etiolated' cells [2]. While the light-grown cells appeared to be similar to the wild-type cells, novel PLB-like ultrastructures were observed only in the dark-grown etiolated cells. The etiolated YFC2 cell has the ability to green upon illumination [2]. During the greening process, the rates of Pchl decrease and Chl formation in YFC2/P202 were almost the same as those of the control cells, suggesting that the overexpressed LPOR does not contribute in the greening process. This was supported by the *in-vitro* LPOR assay of the crude extract prepared from etiolated YFC2/P202 in which LPOR activity was dependent on the exogenously added NADPH. These results suggested that the cyanobacterial LPOR retains the ability to form novel PLB-like aggregates in the presence of Pchl while it does not form the photoactive ternary complex, which is consistent with the previous work [3].

[1] Reinbothe C. et al. (2010) Trends Plant Sci 15: 614-624; [2] Kada S. et al. (2003) Plant Mol Biol 51: 225-235; [3] Masuda S. et al. (2009) Plant J 58: 952-960

## P53: Insights into the functions of flavodiiron proteins in *Synechocystis* sp. PCC 6803

Henna Silén, Luca Bersanini, Natalia Vorontsova, Pengpeng Zhang, Marion Eisenhut, Natalia Battchikova, Yagut Allahverdiyeva, and Eva-Mari Aro

Laboratory of Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

Flavodiiron proteins (FDPs) function in O<sub>2</sub> and NO detoxification in anaerobic prokaryotes [1]. FDP genes have also been identified in certain oxygenic photosynthetic bacteria and eukaryotes [2]. The genome of the cyanobacterium *Synechocystis* sp. PCC 6803 contains four genes encoding FDPs: *sl1521* (Flv1), *sl0219* (Flv2), *sl0550* (Flv3) and *sl0217* (Flv4) [3]. Flv1 and Flv3 proteins have been demonstrated to function in photoreduction of O<sub>2</sub>, known as Mehler-like reaction [3, 4]. Thus, cyanobacteria are able to transfer electrons from the reduced side of PSI to O<sub>2</sub> by means of the Flv1 and Flv3 proteins without formation of reactive oxygen species (ROS). The knock-out mutants of Flv2 and Flv4, unlike mutants in Flv1 and Flv3, are able to perform the photoreduction of O<sub>2</sub>, thus indicating an alternative function for these FDPs. The *flv4-flv2* operon, encoding the Flv4, Sll0218 and Flv2 proteins, is strongly induced by inorganic carbon (Ci) limitation [2]. Moreover,  $\Delta flv2$  and  $\Delta flv4$  mutants have been found to be more susceptible to high light (HL) induced photoinhibition of PSII than wild type (WT) or  $\Delta flv1$  and  $\Delta flv3$ .

Recent studies from our laboratory indicate formation of Flv2/Flv4 heterodimer that binds to thylakoid membranes in light and is involved in a novel electron transfer pathway by receiving electrons from PS II [5]. Flv2/Flv4 might be involved in coupling of phycobilisomes (PBS), the major light harvesting antenna in cyanobacteria, to PS II, thus mediating electron transfer from antenna to PS II reaction center [5]. The thylakoid membrane bound protein Sll0218 functions partially independently of Flv2/Flv4. It is suggested that Sll0218 is stabilizing the PS II dimer in low Ci condition [5]. Interestingly, the Sll0218 and Flv2/Flv4 proteins are differently regulated under Ci limiting condition in phycobilisome-less (PAL) mutant. To further characterize the role of FDPs, *Synechocystis* WT and mutants deficient in different photosynthetic genes were studied under various conditions. We have also studied possible role of Flv2 and Flv4 proteins under Fe-starvation.

The structure of many purified FDPs has been solved by X-ray crystallography [1], but the structure of FDPs in *Synechocystis* and other oxygenic photosynthetic organisms remain uncharacterized. We have constructed overexpression strains in *E. coli* and isolated recombinant FDPs in order to perform detailed spectroscopical characterization of *Synechocystis* FDPs.

[1] Vicente J, Carrondo M, Teixeira M, and Frazão C (2008). Structural studies on flavodiiron proteins. Methods Enzymol. 437:3-19; [2] Zhang P, Allahverdiyeva Y, Eisenhut M, and Aro E-M (2009). Flavodiiron proteins in oxygenic photosynthetic organisms: photoprotection of photosystem II by Flv2 and Flv4 in *Synechocystis* sp. PCC 6803. PLoS One 4:e5331; [3] Helman Y, Tchernov D, Reinhold L, Shibata M, Ogawa T, Schwarz R, Ohad I, and Kaplan A (2003). Genes encoding A-Type flavoproteins are essential for photoreduction of O<sub>2</sub> in cyanobacteria. Current Biology 13:230-235; [4] Allahverdiyeva Y, Ermakova M, Eisenhut M, Zhang P, Richaud P, Cournac L, and Aro E-M (2011). Interplay between flavodiiron proteins and photorespiration in *Synechocystis* sp. PCC 6803. J. Biol. Chem., 286:24007-24014; [5] Zhang P, Eisenhut M, Brandt A-M, Carmel D, Silén H, Vass I, Allahverdiyeva Y, Salminen T A, and Aro E-M (2012). Operon *flv4-flv2* provides cyanobacterial photosystem II with flexibility of electron transfer. Plant Cell (accepted).

## P54: Functional analysis of a conserved histidine kinase, Hik2, in *Synechocystis* using a chimeric protein system

Tomonori Kotajima<sup>1</sup>, Yoshihiro Shiraiwa<sup>1</sup>, and Iwane Suzuki<sup>1</sup>

<sup>1</sup>Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan

Histidine kinases (Hiks) are sensory proteins involved in the perception of extra- or intra-cellular environmental changes and are highly conserved among bacteria, fungi, protists and plants. We have comprehensively investigated functions of histidine kinases in the cyanobacterium *Synechocystis* sp. PCC 6803 by characterization of phenotypes of the mutant of each gene for Hik[1]. However, there were three histidine kinases, Hik2, Hik11, and Hik26, which are functionally unknown because of the indispensability of the genes from the chromosome under the standard growth conditions. Among the three Hiks, Hik2 orthologues are very well-conserved in the genome of cyanobacteria whose genomic DNA had been sequenced. Generally, Hik consists of two functional domains, the signal-input domain located at the N-terminal and the kinase-domain located at the C-terminal. Amino acid sequences of the kinase domains are conserved among the Hiks and these of signal-input domain are diverse to perceive specific stimuli. Hik2 possesses a so-called GAF domain in the signal input domain[2]. Here, we attempted to characterize one of these crucial histidine kinases, Hik2, by introducing an artificial gene encoding a fused sensor protein which has a signal-input domain of Hik2 and a kinase domain of SphS, that is a phosphate-deficiency sensor in cells of *Synechocystis*. We replaced the coding region of the *sphS* gene on the chromosome with the gene for fused sensor. It allowed us to evaluate the sensor activity *in vivo* as the activity of alkaline phosphatase (AP), which is originally regulated by SphS.

The cells possessing the fused sensor had slight activities of AP under the standard growth conditions. Although salt stress due to NaCl induced the AP activity in a dose-dependent manner, other stresses, i.e. upward or downward shifts in temperature, high light and osmotic, did not. We analyzed the effects of several salt compounds on the induction of the AP activity, and found that signal-input domain of Hik2 was related to responsiveness to the concentration of Cl<sup>-</sup> in the external environment. Replacements of the conserved 7 amino acids in the signal-input domain of Hik2 in the chimeric sensor lost the response to salt stress. Bacterial two-hybrid analyses indicated that the GAF domain from Hik2 associated each other in *Escherichia coli* cells, although affinity of the association did not clearly changed due to the changes in Cl<sup>-</sup> concentrations. These results indicated that Hik2 might be a chloride-sensing Hik in the cyanobacteria.

[1] Murata, N., Suzuki, I. Exploitation of genomic sequences in a systematic analysis to access how cyanobacteria sense environmental stress. *J. Exp. Bot.* (2006) 57, 235-247; [2] Galperin, M.Y., Nikolskaya, A.N., Koonin, E.V. Novel domains of the prokaryotic two-component signal transduction systems. *FEMS Microbiol. Lett.* (2001) 203, 11-21.

## P55: Photoprotective role of photosystem I trimer formation as seen by changes in carotenoid composition and energy transfer from phycobilisome to photosystem I reaction center

Kinga Kłodawska<sup>1</sup>, Przemysław Malec<sup>1</sup>, Mihály Kis<sup>2</sup>, Zoltán Gombos<sup>2</sup>, Kazimierz Strzałka<sup>1</sup>

<sup>1</sup>Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 30 – 387 Krakow;

<sup>2</sup>Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, 6701, Szeged, Hungary

Cyanobacterial photosynthetic apparatus is organized in a plant-like manner. The two photosystem complexes are connected by a chain of peptide and low molecular mass electron carriers. Main light harvesting antenna system comprises of phycobilisomes, which transfer excitation energy to photosystem reaction centers. Photosystem I and photosystem II are of a similar, but not identical subunit composition as in higher plants. Cyanobacterial photosystem I exhibits a unique capability of oligomerization into trimeric supercomplex. This process occurs only in the presence of the PsaL subunit of photosystem I. The ratio of monomeric to trimeric PS I complexes in thylakoid membranes is temperature dependent and increases with growth temperature decrease.

Study with the use of a wild type *Synechocystis* PCC6803 and a trimerless mutant strain Δ*psaL* were conducted to clarify physiological significance of photosystem I oligomer formation. Carotenoid pattern changes upon temperature stress in both strains were followed. Absence of photosystem I trimers results in elevated carotenoid content in mutant cells, the most pronounced being that of myxoxanthophyll. Similar response had been reported previously for various stress conditions.

Energy transfer from phycobilisome to photosystem reaction centers was investigated by room-temperature and low-temperature fluorescence spectroscopy. Significant shifts in the emission spectra maxima were observed for monomer only and monomer and trimer containing *Synechocystis* strains.

We conclude that the trimerization of photosystem I decreases the need for photoprotective carotenoid species in cyanobacterial cells. Energy transfer from phycobilisome to photosystem reaction center is altered by the photosystem I oligomerization.

## P56: Quantitative and Functional Characterization of the Hyper Conserved Protein Found in *Prochlorococcus* and marine *Synechococcus*

Jackie Zorz<sup>1</sup>, Katrina DeZeeuw<sup>1</sup>, Olga Zhaxybayeva<sup>2</sup>, and Amanda Cockshutt<sup>1</sup>

<sup>1</sup>Department of Chemistry and Biochemistry, Mount Allison University, Sackville NB, Canada; <sup>2</sup>Department of Biology, West Virginia University, Morgantown WV, USA

An extremely conserved hypothetical protein known as HCP has been found in the ecologically relevant cyanobacterial genera of *Prochlorococcus* and marine *Synechococcus* [1]. Previous work on HCP has shown that it is essentially 100% conserved at the amino acid level and is found in the genomes of all fully sequenced *Prochlorococcus* and marine *Synechococcus* strains [1]. Because these bacteria have significantly reduced genomes with high mutation rates, the conservation of this sequence suggests that this protein plays an important role in the cell [2]. The primary goal of the current study was to determine if HCP was expressed at the protein level by using a previously developed HCP specific antibody. An alternate aim of this work was to determine absolute amounts of photosynthetic protein complexes to gain insight into the thylakoid physiology of these strains. The technique of quantitative immunoblotting was performed to accomplish both of these goals as it was used to obtain absolute protein quantities of HCP along with a variety of photosynthetic proteins. The absolute amounts of various photosynthetic protein complexes were measured and subsequently compared across complexes and across strains. In this manner, it was discovered that of the three strains tested, the ratio of PSI to PSII was the highest at 8:1 in the low light strain *Prochlorococcus* MIT9313 and lowest at 2:1 in the high light strain *Prochlorococcus* Med4. Also the RUBISCO to PSII ratio was lowest at 0.5:1 in the low light strain *Prochlorococcus* MIT9313. It was found that HCP was detected at the protein level in all three strains tested; *Prochlorococcus* sp. MIT9313 and Med4, as well as *Synechococcus* WH8102. Interestingly, through these immunoblots it was discovered that HCP was present at two molecular weights – the expected molecular weight, and at a molecular weight that was twice the expected size. Sequence analysis ruled out upstream start codons as likely contributors to larger than expected HCP sizes. The nature of these higher molecular weight cross-reactive proteins is currently under investigation.

[1] Zhaxybayeva, O., Gogarten, J. P., & Doolittle, W. F. 2007. A hyperconserved protein in *Prochlorococcus* and marine *Synechococcus*. *FEMS Microbiology Letters*. 274: 30-34; [2] Partensky F., Garczarek L. 2010. *Prochlorococcus*: Advantages and Limits of Minimalism. *Annual Review of Marine Science*. 2: 305-331.

## P57: RNA-seq transcriptomics, HetR ChIP-seq, and anti-*nblA* in *Anabaena* PCC 7120

Britt L. Flaherty, David B. Johnson, Neil K. Raina, and James W. Golden

Division of Biological Sciences, University of California San Diego, La Jolla, California, USA

The filamentous cyanobacterium *Anabaena* sp. strain PCC 7120 differentiates specialized nitrogen-fixing cells, called heterocysts, in a developmental pattern along filaments of photosynthetic vegetative cells in response to nitrogen deprivation. Heterocysts differentiate morphologically and physiologically. They shut down oxygen-evolving photosynthesis, degrade their phycobilisomes, and express oxygen-sensitive nitrogenase. Products of nitrogen fixation, probably in the form of glutamine and other amino acids, are provided to nearby vegetative cells. Vegetative cells continue photosynthesis and pass the products of carbon fixation to heterocysts. We have employed directional RNA-seq and ChIP-seq technologies on the Illumina platform to assess changes in RNA abundance in response to nitrogen deprivation and heterocyst development to define changes to the transcriptome and identify targets of key DNA binding proteins in *Anabaena* PCC 7120. Our RNA-seq work mapped each transcript and its abundance at 0, 6, 12, and 21 hours after nitrogen deprivation. The data allowed us to identify new genes and ncRNAs potentially involved in heterocyst development and nitrogen fixation. Our studies have identified antisense RNAs regulated in the response to nitrogen deprivation, including *anti-nblA*, which may be involved in regulation of expression of the phycobilisome degradation protein, NblA. Using chromatin pull-down coupled with deep sequencing, we identified potential new targets for two key transcription factors involved in heterocyst development: HetR, the "master regulator" of heterocyst development, and DevH, a key protein involved in formation of the heterocyst-specific cell wall. We are following up the ChIP-seq studies with *in vitro* and *in vivo* work on the newly identified HetR targets to confirm the ChIP-seq data and eventually determine the functions of these genes as part of the HetR regulon.

## P58: Copper homeostasis in the cyanobacterium *Synechocystis* sp PCC 6803.

Joaquín Giner-Lamia, Luís López-Maury and Francisco J. Florencio.

<sup>1</sup>IBVF - Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Avda Américo Vespucio 49. 41092-Sevilla, SPAIN

Copper is an element required for essential biological processes such as respiration, through the cytochrome oxidase, or in photosynthesis through the electron transfer protein plastocyanin in plants, some algae and cyanobacteria. Copper is also required as metal cofactor on the active sites of different enzymes including oxidases, monooxygenases, dioxygenases and superoxide dismutases. However, if copper is unbound inside the cell, it could be highly toxic, largely due to its ability to catalyze Fenton-like reaction, causing the production of highly reactive hydroxyl radicals that damage biomolecules such as DNA, proteins, and lipids [1]. Hence organisms have developed homeostatic mechanisms to tightly regulate its acquisition, sequestration and efflux. We have characterized a two-component system (CopRS) that is essential for copper resistance in *Synechocystis* sp PCC 6803. The CopRS system regulates the expression of a HME-RND efflux system (CopBAC) and its own expression, in response to the presence of copper in the culture medium, mutants in any of the *cop* genes render cells more sensitive to the presence of copper. To our knowledge this is the first copper resistance system described in cyanobacteria.

This work has been financed by FEDER from EU and grant BFU2010-15708 and by plan E (IEO-USE).

[1] Imlay, J.A. (2003). Pathways of oxidative damage. *Annu Rev Microbiol* 57, 395-418.

## P59: The FtsH3 protease is essential for the induction of IsiA synthesis during iron starvation in the cyanobacterium *Synechocystis* PCC 6803

Vendula Krynicka<sup>1,2</sup>, Martin Tichy<sup>1,2</sup>, and Josef Komenda<sup>1,2</sup>

<sup>1</sup>Institute of Microbiology, Trebon, Czech Republic; <sup>2</sup>Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

The genome of the cyanobacterium *Synechocystis* PCC 6803 contains four genes encoding homologues of the bacterial FtsH protease designated FtsH1-4. Two of them, FtsH1 and FtsH3, are indispensable for cell viability and their crucial function still remains unknown. The physiological role of FtsH3 protease, encoded by *ftsH3* (*slr1604*) gene, was studied using conditional knock-out mutant SynFtsH3reg, in which the *ftsH3* gene is expressed under promoter stringently regulated by availability of ammonium ions. As the cellular level of FtsH proteases generally increases in response to various stresses, we further tested the mutant for its adaptability to nutrient deficiency. Specifically the iron deficiency strongly impacts photosynthetic apparatus in cyanobacteria, and it leads to various structural and functional changes in the photosynthetic membrane complexes. The FtsH proteases can be involved in such processes and for this reason we were interested in whether FtsH3 plays any role in the adaptation to iron depletion stress.

The wild type strain of *Synechocystis* PCC 6803 is able to adapt to this stress by expression of IsiA protein functioning as a chlorophyll storage protein and/or as an antenna for PSI. Significant changes in fluorescence and absorption properties of the cells accompany this process. In contrast, the SynFtsH3reg mutant downregulated by ammonium ions exhibited no changes typical for iron starvation, including minimal expression of IsiA.

IsiA expression is controlled at both transcriptional and post-transcriptional level in cyanobacteria and we initially compared the *isiA* transcript level during iron starvation in WT and SynFtsH3reg downregulated cells using RT PCR. In the WT cells the transcript level for IsiA largely increased while no such increase was detected in the mutant. These results indicated that FtsH3 affects the transcriptional regulation of IsiA. Transcription of the *isiA* gene is controlled by Fur, a transcriptional repressor, which is bound to the *isiA* promoter. In the absence of iron Fur is released and probably degraded. The possibility that FUR could be a substrate for FtsH3 was tested by protein analyses. The results provided evidence that the cells deficient in FtsH3 accumulate FUR protein. It probably remains partially bound to DNA and is not degraded even in the absence of iron and therefore the *isiA* gene is not expressed.

## P60: Up-regulation of photoprotective mechanisms makes the $\Delta$ sigCDE strain of *Synechocystis* sp. PCC 6803 tolerant against photoinhibition

Kaisa Hakkila<sup>1</sup>, Taras Antal<sup>2</sup>, Esa Tyystjärvi<sup>1</sup> and Taina Tyystjärvi<sup>1</sup>

<sup>1</sup>Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland; <sup>2</sup>Faculty of Biology, Moscow State University, Moscow, Russia

Cyanobacteria respond to both light intensity and quality by adjusting the expression of many genes. In sub-optimal environmental conditions, sigma factors of the bacterial RNA polymerase play central regulatory roles by determining preferable promoters that the RNA polymerase holoenzyme binds to initiate transcription. *Synechocystis* sp. PCC 6803 genome encodes nine  $\sigma$  factors. The housekeeping  $\sigma$  factor, SigA, is essential for cell viability. Group 2  $\sigma$  factors, SigB, SigC, SigD and SigE, are important for acclimation to different environmental conditions. Group 3  $\sigma$  factors, SigF, SigG, SigH and SigI are structurally different and they have distinct roles in cell function.

The  $\Delta$ sigCDE strain (SigB is the only functional group 2  $\sigma$  factor) lost light-saturated PSII activity in bright light more slowly than the control strain. The PSII repair cycle was as efficient in the  $\Delta$ sigCDE strain as in the control strain, but the light-induced damage of PSII occurred more slowly in the  $\Delta$ sigCDE strain. In isolated thylakoids, similar photoinhibition rates were measured for the  $\Delta$ sigCDE and control strains, and biophysical measurements revealed that electron transfer reactions occurred similarly in both strains. This suggested that the resistance of the  $\Delta$ sigCDE strain to photoinhibition is not due to differences in PSII properties, but some photo-protective mechanisms functioning very efficiently in the  $\Delta$ sigCDE strain.

Analysis of possible photoprotective mechanisms showed that few of them were upregulated in the  $\Delta$ sigCDE strain while others were not. HPLC analysis revealed that the  $\Delta$ sigCDE strain had a higher carotenoid content than the control strain. Specifically, the  $\Delta$ sigCDE strain had more myxoxanthophyll and zeaxanthin than the control strain while the amounts of echinenone and  $\beta$ -carotene were similar. Non-photochemical quenching, a mechanism that dissipates excitation energy of the phycobilisomes to heat, was higher in the  $\Delta$ sigCDE strain than in the control strain. Furthermore, the photoprotective *flv4-sll2018-flv2* operon was up-regulated in the  $\Delta$ sigCDE strain. No differences were detected in state transitions, IsiA protein, or in PSII charge recombination reactions.

## P61: *In vivo* reconstitution of circadian system by heterologously expression of the cyanobacterial Kai proteins in *Escherichia coli*

Sayuri Abe, Koya Matsuda, Katsuaki Oyama, Kazuki Terauchi

Department of Life Sciences, Ritsumeikan University, Kusatsu, Shiga, Japan

The circadian clock is a basic cellular system used by almost all organisms to adapt their metabolism and behavior to day-night alternation. Cyanobacteria are the simplest organisms that exhibit circadian rhythms. The cyanobacterial circadian oscillator composed of only three proteins, KaiA, KaiB, and KaiC sustains an ordered pattern of KaiC phosphorylation. KaiA enhances the KaiC phosphorylation and KaiB antagonizes the KaiA action. KaiC is rhythmically assembled and disassembled with KaiA and KaiB, resulting in periodical formation of hetero-complexes of the three Kai proteins. *In vitro* reconstitution of the KaiC phosphorylation cycle, achieved by simple mixing of the three Kai proteins with ATP [1] provided a means of studying the detailed mechanisms of the Kai-proteins oscillator. DNA microarray analysis revealed that the expressions of many genes showed the circadian rhythm in the cyanobacterium *Synechococcus elongates* PCC 7942 [2]. However, the mechanism by which the Kai-based circadian clock controls diurnal genome-wide transcription in the cells remains to be solved. In this study, we examined whether the cyanobacterial circadian clock composed of Kai proteins operate in a heterologous host. Three *kai* genes of the cyanobacterium *Synechococcus elongates* PCC 7942 were introduced to *Escherichia coli*, and KaiA, KaiB, KaiC proteins were co-expressed in the cells. The *E. coli* cells were grown in continuous cultures at 30 degrees C. Three Kai proteins were detected immunologically in the crude extract of *E. coli*. The phosphorylated and unphosphorylated forms of KaiC were discriminated by the slight mobility change in SDS-PAGE profiles and the ratios of phosphorylated form to the total KaiC were estimated to evaluate the circadian oscillation of the KaiC phosphorylation states. The ratios were kept in constant in the *E. coli* cells expressing only the *kaiC* gene. On the other hand, the ratios were chronologically oscillated in the *E. coli* possessing three *kai* genes. These results suggested that a circadian clock is reconstituted in the cells by heterologous co-expression of *kaiA*, *kaiB* and *kaiC*. This synthetic approach for generation of a circadian clock in *E. coli* might provide a molecular basis for understanding the regulation of the gene expression in a periodic manner in the cells.

[1] Nakajima M. *et al.* (2005) *Science* 308, 414-415; [2] Ito H. *et al.* (2009) *Proc Natl Acad Sci USA* 106, 14168-14173.

## P62: Effects of temperature and nitrate concentration on growth and morphology of *Anabaenopsis elenkinii* Miller (Cyanobacteria) isolated from the alkaline shallow lake of the Brazilian Pantanal

Kleber R. S. Santos<sup>1, 2</sup> and Célia L. Sant'Anna<sup>1</sup>

<sup>1</sup>IBT- Nucleus of Phycology, Institute of Botany, 04301-902, São Paulo, Brazil; <sup>2</sup>PhD Student, Institute of Botany, Brazil, grant support of FAPESP (Process 2009/51655-1) santoskrs@gmail.com

*Anabaenopsis elenkinii* Miller normally occurs in alkaline waters from tropical regions and is the dominant species in the alkaline shallow lakes of the Brazilian Pantanal [1]. In this study, we analyzed the effects of temperature and nitrate concentration (NaNO<sub>3</sub>) on the growth rate (cells.mL<sup>-1</sup>), biovolume (mm<sup>3</sup>.L<sup>-1</sup>) and trichome morphology of the *A. elenkinii* CCIBt1059 strain isolated from an alkaline lake. The experiments were carried out in growth chamber (*n* = 3) for 30 days under modified medium BG-11 (3% NaNO<sub>3</sub>), pH 9.5, photoperiod 12–12 light–dark cycle, irradiance of 80–100 μmol photons m<sup>-2</sup> s<sup>-1</sup> and temperature 25°C as a control condition. The analyzed treatments were the following: temperature 30°C and 35°C and nitrate concentrations 50% and 0%. The applied statistical analyses were ANOVA, Tukey test and RDA. The higher values of growth rate ( $\mu$ ) and cell yield (*R*) were observed at 50% nitrate concentration. However, at 0%, these parameters were greater than in the control condition, probably related to the efficient capacity of the strain to fix atmospheric nitrogen. This fact is evidenced by the higher frequency of heterocytes in 0% nitrate concentration (*p* > 0.05) when compared with the control condition and the 50% nitrate concentration. In relation to temperature, the growth rate was higher at 30°C. However, the highest cell yield was observed at 25°C and growth was inhibitory at 35°C. The morphometric variability within each treatment between exponential and stationary growth was clearly higher than that in the different treatments. In most cases the higher values of width of the vegetative cell and heterocytes were observed in exponential growth. Our results showed that optimum temperature and nitrogen concentration for *A. elenkinii* are 25°C and 50% NaNO<sub>3</sub> respectively. Furthermore, they indicate that with increased temperature the growth limitation can occur in terms of density of cells and biomass, and in the context of global warming, reduced growth of *A. elenkinii* could become an important concern for biological processes in these alkaline lakes.

[1] Santos, K.R.S., Jacinavicius, F.R. & Sant'Anna, C.L. (2011). Effects of the pH on growth and morphology of *Anabaenopsis elenkinii* Miller (Cyanobacteria) isolated from the alkaline shallow lake of the Brazilian Pantanal. *Fottea* 11(1): 119-126.



### P63: Influence of enhanced CO<sub>2</sub> concentration on regulation of photosynthesis in diazotrophic cyanobacteria

**Kristina Felcmanova<sup>1,2</sup>, Barbora Sediva<sup>1</sup>, Dana Luhanova<sup>2</sup>, Ondrej Prasil<sup>1</sup>**

<sup>1</sup>Institute of Microbiology, Academy of Sciences of the Czech Republic, Laboratory of Photosynthesis, CZ-37981 Trebon, Czech Republic, felcmanova@alga.cz; <sup>2</sup>University of South Bohemia, Faculty of Science, CZ-37005 Ceske Budejovice, Czech Republic

Diazotrophic cyanobacteria are important contributors to ocean primary production and they are important providers of new nitrogen to the oligotrophic ocean areas. Increasing atmospheric concentration of CO<sub>2</sub> has influence on the ocean carbonate chemistry, on its acidification and on phytoplankton physiology. Several reports have studied cyanobacterium *Trichodesmium* sp., which responds to elevated concentration of CO<sub>2</sub> with increase of its growth rate. We therefore hypothesized that high pCO<sub>2</sub> might be beneficial for all diazotrophs. We examined the physiological response, growth rate, carbon and nitrogen fixation activity of two diazotrophic cyanobacteria with different strategies of nitrogen fixation: unicellular *Cyanothece* sp. ATCC 51142 and filamentous heterocyst-forming *Anabaena* sp. PCC 7120 to current (390ppm) and future (900ppm) atmospheric CO<sub>2</sub> concentration. Only few data are available reporting the response of diazotrophic cyanobacterium *Anabaena* sp. and so far no experiments have been done with cyanobacterium *Cyanothece* sp. and its response to the elevated CO<sub>2</sub>. High pCO<sub>2</sub> increased the growth rate in *Cyanothece* sp. by 32%, however in cyanobacterium *Anabaena* sp., the growth rate was inhibited by 34%. Nitrogenase activity was stimulated under elevated CO<sub>2</sub> in both species. The total nitrogen fixation derived from nitrogenase activity was under high pCO<sub>2</sub> 2-fold higher in cyanobacterium *Cyanothece* sp. and 1.5-fold higher in cyanobacterium *Anabaena* sp. in comparison with cultures cultivated at current pCO<sub>2</sub>. We also observed that cyanobacterium *Cyanothece* sp. fixes nitrogen at the end of light phase, although it has been assumed that nitrogen fixation in unicellular cyanobacteria is restricted only to the dark phase. In summary, our results do not confirm the initial hypothesis. Possible explanations for the contrasting response among *Cyanothece* and *Anabaena* may be found in different morphological and ecological strategies of diazotrophic cyanobacteria.

### P64: Mutation study of the PatS signaling peptide in *Anabaena* sp. PCC 7120

**Laura Corrales-Guerrero, Vicente Mariscal, Enrique Flores and Antonia Herrero**

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, E-41092 Sevilla, Spain

The process of heterocyst differentiation requires a complex regulation in which a large number of proteins are involved [1]. The PatS peptide, first identified in *Anabaena* sp. PCC 7120, is a negative element of the process that is expressed in some cells of the filament and inhibits the differentiation of neighboring cells [2]. Inactivation of the *patS* gene leads to a Multiple Contiguous Heterocyst (Mch) phenotype, whereas overexpression of *patS* produces a total inhibition of differentiation [2]. It has been proposed that PatS or a PatS-derivative could be transferred through the filament by an unknown mechanism. The *patS* ORF could encode a 17 or a 13 amino-acid peptide depending on which of two possible methionine codons is used for translation initiation. The C-terminal part of PatS is essential for activity and, when added to the external medium, inhibits the process of differentiation [3]. The N-terminal region of PatS is conserved in several heterocyst-forming cyanobacteria, supporting the possibility that this region is involved in the export of the peptide as occurs in other bacterial cell-cell signaling systems. We have studied the site of PatS translation initiation by fusion of the *gfp* gene at different positions of *patS* and mutagenesis. GFP fluorescence emission was observed when the *gfp* gene was inserted in phase after the first or the second methionine codon. However, substitution of each methionine codon by an alanine codon showed that the strain bearing PatS with the second methionine mutated has a wild-type phenotype, whereas the strain bearing PatS with the first methionine mutated presents a phenotype similar to that of a *patS* deletion mutant. These results show that *patS* is translated from the first possible methionine codon in the DNA sequence. We have also addressed the possible processing of the PatS peptide by studying the phenotype of *Anabaena* strains producing modified PatS peptides bearing GFP or 6-His tag fusions as well as amino acid substitutions in each residue of the peptide. Insertions in the N-terminal region of the gene led to a reduction of the number of heterocysts, with an almost total inhibition in the case of the insertion of the GFP after the second methionine. Substitution of V<sup>7</sup> also abolished differentiation. These results can be interpreted assuming an important role of the N-terminal part of the peptide in PatS processing so that the non-processed peptide accumulates in the producing cell inhibiting its differentiation. The substitutions R<sup>13</sup>A, G<sup>16</sup>A and R<sup>17</sup>A resulted in a phenotype similar to that of the *patS* deletion mutant, and substitutions E<sup>12</sup>A, G<sup>14</sup>A and S<sup>15</sup>A increased the frequency of heterocysts, corroborating the importance of the C-terminal part of the peptide for PatS activity.

[1] Flores E. & Herrero A. (2010) Nat Rev Microbiol 8:39-50; [2] Yoon H.S. & Golden J.W. (1998) Science 282:935-38; [3] Wu X. et al. (2004) J Bacteriol 186: 6422-29



## P65: Inactivation of SepJ permease domain homologues in *Synechocystis* and *Synechococcus*

**Leticia Escudero, Mireia Bumat, Vicente Mariscal, Antonia Herrero and Enrique Flores.**

Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC and Universidad de Sevilla, E-41092 Sevilla, Spain

Multicellularity implies at least three processes: cell-cell adhesion, intercellular communication and cellular differentiation. Heterocyst-forming cyanobacteria, such as *Anabaena* sp. strain PCC 7120, grow under nitrogen limitation as multicellular filaments where two metabolically complementary cell types coexist. The vegetative cells perform the photosynthetic fixation CO<sub>2</sub> and heterocysts are specialized in N<sub>2</sub> fixation [1]. SepJ and FraC/FraD proteins have been shown to be involved in cell-cell adhesion and intercellular communication in *Anabaena* sp. strain PCC 7120 [2, 3]. SepJ is a membrane protein located at the cell poles in the intercellular septa of the filament. It has three different domains: a periplasmic N-terminal domain with strongly predicted coiled-coil structures, a linker domain and an integral membrane C-terminal permease domain. Mutants carrying SepJ proteins with specific fragment deletions or hybrid (*Anabaena-Trichodesmium erythraeum*) SepJ proteins have permitted to identify an essential role of the coiled-coil domain in subcellular localization, intercellular molecular transfer and diazotrophy, whereas the permease domain appears to provide a specific function for diazotrophy [2]. A SepJ protein bearing three different domains is characteristic to the filamentous heterocyst-forming cyanobacteria. However, similar proteins bearing only the coiled-coil and permease domains are found in filamentous non-heterocyst-forming cyanobacteria, and proteins homologous to the permease domain are encoded in the genomes of many unicellular cyanobacteria. The latter can be a tool for gaining information on the SepJ permease domain, which shows homology to permeases of the DMT superfamily that export amino acids, other metabolites such glucose or drugs [4]. *Synechocystis* sp. PCC 6803 and *Synechococcus* sp. PCC 7942 are unicellular cyanobacteria bearing genes, *sll1319* and *synpcc7942\_1024*, respectively, encoding homologues to the *Anabaena* SepJ permease domain. In order to analyse phenotypic traits associated to SepJ permease function, deletion mutants of *sll1319* (strain CSMI18) and *synpcc7942\_1024* were constructed. Glutamate transport studies and tests of resistance/sensitivity to drugs were performed in strain CSMI18 but no changes with respect to the wild-type strain were observed. To complement this approach, we have addressed the heterologous expression of *Anabaena* SepJ in the *synpcc7942\_1024* mutant of *Synechococcus* sp. PCC 7942 and in different *E. coli* strains. The resulting constructs are currently being characterised.

[1] Flores E. & Herrero A. (2010) Nat. Rev. Microbiol. 8:39-50; [2] Mariscal V. et al. (2011) Mol. Microbiol. 79:1077-1088; [3] Merino-Puerto V. et al. (2011) Mol. Microbiol. 82:87-98; [4] Jack D.L. et al. (2001) J. Biochem. 268:3620-3639;

## P66: The omega subunit of RNA polymerase is essential for *Synechocystis* sp. PCC 6803 in heat but not in salt stress

**Liisa Gunnelius, Kaisa Hakkila, and Taina Tyystjärvi**

Molecular Plant Bioogy, Department of Biochemistry and food chemistry, University of Turku, Turku, Finland

The bacterial RNA polymerase is responsible for the transcription of all kinds of RNA. The catalytical core is built around the two large subunits, beta and beta-prime, with two identical alpha subunits enhancing the reaction rate and a tiny omega subunit assisting beta-prime in folding and assembly to the complex. In cyanobacteria, the beta-prime subunit is split and the part corresponding to the N-terminal half in other bacteria is called gamma, whereas cyanobacterial beta-prime corresponds only to the C-terminal part of the beta-prime in other bacteria. For promoter-specific initiation, the core recruits one sigma factor subunit.

The *rpoZ* gene of the cyanobacterium *Synechocystis* sp. PCC 6803, encoding the omega subunit of RNA polymerase, was interrupted with a kanamycin resistance cassette. The mutant strain grew as well as the control strain in our standard conditions: BG-11 medium, pH 7.5, normal air, continuous illumination at the photosynthetic photon flux density of 40 micromoles per second per square meter, and 90 rpm shaking at +32C. At +40C, the control strain grew slightly better than at +32C but the *rpoZ* mutant died within the first 24 hours. Increasing the availability of inorganic carbon at +40C by raising the pH of the medium to 8.3 did not enhance the growth of the *rpoZ* mutant, although some other heat-sensitive mutants including sigma factor inactivation strains benefit from the excess carbon [1]. Interestingly, lowering the temperature to +38C restored the growth of the mutant almost to the level of the control strain. In moderate salt stress (0.7M NaCl) the growth of the *rpoZ* mutant was very slow, but the cells were proliferating during the whole observation time (96 hours). Our results showed increased carotenoid content in mutant cells in standard conditions. Currently we are comparing the transcript profiles of the *rpoZ* mutant and the control strain in standard conditions and in heat stress and the results will be discussed in detail at the meeting.

[1] Gunnelius L, Tuominen I, Rantamäki S, Pollari M, Ruotsalainen V, Tyystjärvi E, Tyystjärvi T (2010) SigC sigma factor is involved in acclimation to low inorganic carbon at high temperature in *Synechocystis* sp. PCC 6803. Microbiology 156:220-229

## P67: Fe-S cluster metabolism in *Synechocystis* sp. PCC6803

Linda Vuorijoki<sup>1</sup> and Patrik R. Jones<sup>1</sup>

<sup>1</sup>Bioenergy Group, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

Iron-sulfur (Fe-S) clusters constitute a versatile group of cofactors that function in various cellular processes including electron transfer, redox sensing and gene regulation. Some iron-sulfur clusters are labile, particularly under oxidative conditions, as oxidation of one of the catalytic irons causes the cluster to degrade. It is therefore intriguing that Fe-S clusters are employed in central metabolic roles in photosynthesis which both evolves O<sub>2</sub> and generates radical oxygen species under conditions of stress. The inevitable formation of the radical species may be one of the reasons why cyanobacteria appear to exclusively rely on the SUF system in Fe-S assembly, which is the most shielded against oxidation [1,2]. In fermentative prokaryotes, SUF plays an ancillary role to the ISC system and has been linked to oxidative stress conditions rather than general Fe-S cluster assembly. In cyanobacteria, the SUF system is regulated by the Fe-S containing negative transcriptional regulator SufR, similar to IscR in fermentative prokaryotes. We were interested to understand the role of SUF regulation on the functionality of Fe-S clusters with a central metabolic role. Previously, it was reported that complete elimination of *sufR* was not possible in *Synechocystis* sp. PCC 6803 [1], suggesting that tight control of the expression of the *suf* operon is essential for survival. However, we were able to generate a fully segregated *sufR* deletion mutant and study the impact on growth and PSII/PSI functionality in relation to light. The *sufR* deletion mutant displayed a shift in the absorbance spectrum and only minor differences in the growth rate compared to wild-type under continuous low light. The gene expression of *sufB*, the first gene of the *suf* operon, was stimulated strongly, as expected, whilst all other tested genes were unaffected (*psaC*, *pgl5*, *sodB*, *perR*). The activity of the native NiFe-hydrogenase was enhanced both under low and high light, whilst only minor differences in P700 redox-kinetics were observed. Surprisingly, the growth rate of the *sufR* mutant was enhanced relative to wild-type under cyclic day-night conditions (day phase peaking at 500 μE) over the first couple of cycles, though no difference was observed at higher cell density in later cycles. In order to unravel the mechanisms of regulation of Fe-S metabolism and to discover the proteins involved in the iron-sulfur cluster assembly and repair in cyanobacteria, further transcriptomic and proteomic approaches will be used in the near future.

[1] Wang, T. et al. 2004. J. Bacteriol. 186(4):956-967; [2] Ayala-Castro, C. et al. 2008. Fe-S cluster assembly pathways in bacteria. Microbiol Mol Biol Rev 72: 110-125.

## P68: Regulation of cell division during heterocyst development in *Anabaena* PCC7120

Loralyn M. Cozy<sup>1</sup>, and Sean M. Callahan<sup>1</sup>

<sup>1</sup>University of Hawaii, Department of Microbiology, 2538 McCarthy Mall, 207 Snyder Hall, Honolulu, HI 96822

Growth during development is critical for multicellular organisms. The appropriate cessation of growth, however, is equally critical to produce the correct arrangement of differentiated tissues. The Gram-negative filamentous cyanobacterium *Anabaena* PCC 7120 provides a model for the study of division during differentiation. *Anabaena* uses fixed nitrogen from the environment to support growth and division. When fixed nitrogen becomes limiting, approximately every tenth cell terminally differentiates into a specialized cell type: the heterocyst, which ceases to divide [1].

Division in bacteria follows a defined series of steps [2]. After replication and segregation of the chromosome, FtsZ is polymerized into the cytokinetic Z-ring at the midcell. Placement of the Z-ring is governed by the Noc and Min systems. Noc ensures that Z-ring constriction does not occur over the chromosome, while Min probabilistically represses Z-ring polymerization at the poles, leaving the midcell open for ring formation. The Min system in Gram-negative bacteria consists of three proteins: MinC, MinD and MinE. MinC and MinD interact along the membrane to inhibit FtsZ polymerization. MinE inhibits MinCD polymerization by repeated oscillations to permit Z-ring formation at the midcell.

Currently, the relationship between cell division and heterocyst differentiation is unclear. When cell division was inhibited chemically or genetically, differentiation was also inhibited, suggesting that division is required for heterocyst development [3,4]. Time-lapse tracking of individual cells, however, showed that some cells in the population that did not undergo division during nitrogen limitation still became heterocysts, suggesting that division is not required immediately prior to differentiation [5].

To clarify the role of the Min system in *Anabaena* division and heterocyst development, *minC* and *minE* were translationally coupled to yellow fluorescent protein (YFP) and placed under control of the copper-inducible *petE* promoter. Subcellular localization of the MinC-YFP and MinE-YFP fusions was observed by confocal fluorescence microscopy in nitrogen replete and limiting conditions. In wild type, overexpression of MinC and MinE perturbed normal cell shape and often appeared as alternating elongated and "mini" cells, suggesting that the fusion proteins were functional. MinC and MinE localized as polar foci. Localization was further assessed in strains individually mutant for the heterocyst development regulators *hetR*, *patA* and *hetF*. This work suggests that the Min system of *Anabaena* may also be related to heterocyst development and lays the foundation understanding cell division in a developmental context.

[1] Kumar, K. et al. (2010) Cold Spring Harb Perspect Biol. a000315; [2] Margolin, W. (2000) FEMS Micro Rev. 24: 531-548; [3] Sakr, S. et al. (2006) J Bact. 188: 1396-1404; [4] Sakr, S. et al. (2006) J Bact. 188: 5958-5965; [5] Asai, H. et al. (2009) PLoS ONE. 4 (10): e7371

**P69: Involvement of *flv4-2* operon in photoprotection of PSII in *Synechocystis* sp. PCC6803****Luca Bersanini, Henna Silen, Pengpeng Zhang, Yagut Allahverdiyeva, Natalia Battchikova and Eva-Mari Aro**

Department of Biochemistry and Food Chemistry, Molecular Plant Biology, University of Turku; e-mail: lucber@utu.fi

The genome of *Synechocystis* sp. PCC 6803 comprises four FDPs (flavodiiron protein) genes: *flv1* (*sll1521*), *flv3* (*sll0550*) and *flv2* (*sll0219*) and *flv4* (*sll0217*), with the last pair organized in the operon together with the *sll0218* gene.

Expression of the *flv2* and *flv4* genes is strongly induced under air level of CO<sub>2</sub> (LC condition) at both the transcript and protein levels. The most rapid induction occurs at LC conditions and high light irradiance<sup>1</sup>. In the  $\Delta flv2$  and  $\Delta flv4$  mutants grown in LC conditions, a strong light-dependent decline in functional PSII centers was observed, suggesting that Flv2 and Flv4 are crucial for protection of PSII centers against photoinhibition<sup>1</sup>.

The Flv2 and Flv4 proteins form a heterodimer which is localized in cytoplasm but also has a high affinity to membrane in the presence of divalent cations. Sll0218 resides in the thylakoid membrane in association with yet unidentified high molecular mass protein complex. Biophysical measurements performed with different deletion mutants provided evidence of a novel electron transfer pathway to the Flv2/Flv4 heterodimer from PSII and an important role of Sll0218 in the stabilization of the PSII dimers<sup>2</sup>.

To further investigate this hypothesis, we overexpressed in *Synechocystis* the native *flv4-sll0218-flv2* operon. In order to reveal possible interacting partners, other overexpression constructs with various affinity tags at the N- and C-termini of genes of the *flv4-2* operon were obtained. Biochemical and biophysical analyses performed with these mutants highlight the important role of Flv4, Sll0218 and Flv2 in photoprotection of PSII.

The *flv4-flv2* operon provides many cyanobacteria with a new type of photoprotective mechanism, which is evolved in parallel with oxygen evolving PSII.

[1] Zhang et al. 2009, Plos One 4(4):e5331; [2] Zhang et al. 2012, Plant Cell, in press.

**P70: A novel rod-membrane linker CpcL in cyanobacteria: Does CpcL form universal photosystem I-specific antenna?****Mai Watanabe<sup>1,2</sup>, Kumiko Kondo<sup>1,3</sup>, Rei Narikawa<sup>1,4</sup> and Masahiko Ikeuchi<sup>1,2</sup>**

<sup>1</sup>Department of Life Sciences (Biology), Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan; <sup>2</sup>Japan Science and Technology Agency (JST), CREST, Saitama, Japan; <sup>3</sup>RIKEN Plant Science Center, Kanagawa, Japan; <sup>4</sup>Science and Technology Agency (JST), PRESTO, Saitama, Japan.

Phycobilisome (PBS) is a major antenna protein complex in cyanobacteria and some algae [1]. It preferentially transfers light energy to photosystem II, whereas photosystem I (PSI)-specific antenna has not been identified. Generally, PBS is composed of several rods and core cylinders, which are connected with a rod-core linker polypeptide CpcG. Previously, we reported that CpcG-like polypeptide (CpcG2) forms unusual PBS and is involved in preferential energy transfer from PC rods to PSI in *Synechocystis* sp. PCC 6803, using specific antibodies and gene disruptants [2,3]. However, there is no evidence for direct connection between CpcG2 and PSI. Recently, we succeeded in isolation of a supercomplex consisting of the PSI tetramer [4] and unusual PBS from *Anabaena* sp. PCC 7120. The supercomplex included PC but not allophycocyanin core. We found that only CpcG3 is present in this supercomplex, whereas other CpcG copies (CpcG1, CpcG2 and CpcG4) were recovered in the classic PBS supercomplex. Notably, the *Synechocystis* CpcG2 and *Anabaena* CpcG3 possess a hydrophobic segment in C-terminus, while the other CpcG copies do not. We raised specific antibodies to discriminate these CpcG and CpcG-like polypeptides and found that the *Anabaena* CpcG3 is tightly associated with thylakoid membrane and recovered exclusively into PSI-PBS supercomplex after mild fractionation. The study of gene disruptant revealed that CpcG3 is critical to form the PBS-PSI supercomplex. These results led us to conclude that the CpcG-like polypeptide having the hydrophobic tail is a novel rod-membrane linker that facilitates energy transfer from the PBS rods to PSI. Hence, we renamed such CpcG-like polypeptide CpcL. Database search revealed that CpcL having the C-terminal hydrophobic tail is widely distributed to various cyanobacteria in addition to the conventional rod-core linker CpcG. Phylogenetic analysis of CpcG and CpcL showed that there are three subgroups of CpcL in cyanobacteria. CpcL of *Anabaena* and *Synechocystis* is clustered into Group I and Group II, respectively. Group III includes CpcL of marine *Synechococcus*. We raised specific antibodies against CpcG and CpcL of marine *Synechococcus* WH 8102 and are now investigating their localization in cells. We will discuss about the universal role of CpcL and CpcL-PBS in cyanobacteria especially for preferential energy transfer to PSI.

[1] Watanabe et al (2012) Biochim Biophys Acta in press; [2] Kondo et al (2005) Photosynth Res 84: 269-73; [3] Kondo et al (2007) Plant Physiol 144: 1200-10; [4] Watanabe et al. (2011) Plant Cell Physiol 52: 162-8.

## P71: Function of Flavodiiron proteins in *Anabaena* PCC 7120

**Maria Ermakova, Natalia Battchikova, Hannu Leino, Yagut Allahverdiyeva and Eva-Mari Aro**

Department of Chemistry and Food Chemistry, Molecular Plant Biology, University of Turku, Finland; e-mail to marerm@utu.fi

Flavodiiron proteins (FDP) (or A-type flavoproteins), involved in detoxification of O<sub>2</sub> or NO in anaerobic Bacteria and Archaea, were found in all so far sequenced cyanobacterial species. Genome of *Synechocystis* PCC 6803 contains four genes encoding FDPs. The Flv1 and Flv3 proteins function in photoreduction of O<sub>2</sub> directly to water (cyanobacteria-specific type of Mehler reaction<sup>1,2</sup>) donating electrons to molecular oxygen on the reducing side of Photosystem I. The Flv2 and Flv4 proteins participate in photoprotection of Photosystem II.<sup>3,4</sup>

Filamentous N<sub>2</sub>-fixing cyanobacteria generally have a bigger gene family encoding the FDPs. Six genes could be found in genome of *Anabaena* sp. PCC 7120. Four of them, *flv1b*, *flv2*, *flv3b* and *flv4* are highly similar to corresponding genes in *Synechocystis*, but the two extra genes, *flv1a* and *flv3a*, are slightly more different from corresponding *flv1* and *flv3* in *Synechocystis*. We attempted to determine the reasons for duplication of the *flv1* and *flv3* genes in *Anabaena*.

The results of RT-qPCR show clear differences in the expression behaviour of the *flv1b* and *flv3b* genes compared to *flv1a* and *flv3a*, respectively, in all experiments performed so far. The expression level of the "b" genes increased upon a shift of cells from high to ambient CO<sub>2</sub> level and also during the high light treatment independently of the source of nitrogen. On the contrary, the transcription levels of the "a" genes depended only on the availability of combined nitrogen in the growth medium, with strong induction occurring upon nitrogen deprivation. Fusion of target proteins with YFP confirmed that Flv1A and Flv3A are localized exclusively in heterocysts, while Flv1B and Flv3B are present in vegetative cells.

We have constructed mutants lacking the *flv1a*, *flv1b*, *flv3a*, and *flv3b* genes.  $\Delta flv1a$  and  $\Delta flv3a$  mutants demonstrated strong phenotype at N<sub>2</sub>-fixing conditions. These mutants demonstrated light-induced oxygen uptake whereas it was completely inhibited in  $\Delta flv1b$  and  $\Delta flv3b$ .

We suggest that the flavodiiron proteins Flv1B and Flv3B perform photoreduction of O<sub>2</sub> in vegetative cells of *Anabaena*, but Flv1A and Flv3A are related to N<sub>2</sub> fixation.

[1] Helman et al., *Current Biology* (2003); [2] Allahverdiyeva et al, *JBC* (2011); [3] Zhang et al., *Plos One* (2010); [4] Zhang et al., *Plant Cell* (2012, in press)

## P72: Structure / Activity Analysis of the Carboxysomal $\gamma$ -Carbonic Anhydrase, CcmM

**Maryam Moazami-Goudarzi, Charlotte de Araujo, and George S. Espie**

Dept. of Cell & Systems Biology, University of Toronto, Mississauga ON Canada

Carboxysomes are complex biochemical compartments essential for photosynthesis and the CO<sub>2</sub> concentrating mechanism in cyanobacteria. These prokaryotic organelles house most of the cellular complement of ribulose 1, 5- biphosphate carboxylase (Rubisco) within a protein shell. CO<sub>2</sub> is delivered to Rubisco by one of a variety of strain-specific HCO<sub>3</sub><sup>-</sup> dehydration complexes (BDC). CcmM is a universal component of the BDC in  $\beta$ -cyanobacteria comprised of an N-terminal  $\gamma$ -carbonic anhydrase (CA) like domain and 3 or 4 repeats of an RbcS-like domain.

Amino acid sequence analysis of the  $\gamma$ -CA like domain from 35 cyanobacterial strains revealed that it can be categorized into two distinct groups. We show that the first group, exemplified by CcmM from *Nostoc* sp PCC7120, is a catalytically active enzyme as determined by <sup>18</sup>O exchange assays. Using truncated forms of CcmM, biochemical analysis and homology modeling we defined the boundary of the  $\gamma$ -CA catalytic domain to reside within the N-terminal 201 amino acids. We also identified several important structural motifs which are unique to the  $\gamma$ -CA domain of the cyanobacterial enzyme. These included the A(7)APPTPWS(14) motif found in the  $\beta$ 1 -  $\beta$ 1 loop, a small  $\alpha$  helix ( $\alpha$ C) between residues 197 and 207 and a disulfide bond that spans  $\alpha$ B-  $\alpha$ C between C194 and C200. Reducing agents, DTT, TCEP and THP inhibited the  $\gamma$ -CA activity of recombinant *Nostoc* PCC7120 CcmM209 through a mechanism that was reversed by the thiol oxidizing agent diamide. These data suggest that the C194-C200 disulfide bond was critical for the oxidative activation and stabilization of enzyme structure [1]. Consistent with this is the observation that the C194S, C200S mutant of CcmM209 was 55% less active.

To further examine the role of disulfide bond, we assayed CcmM 209 from *Synechococcus* PCC7002 for CA activity. This protein is a member of the second group of CcmM proteins that have a divergent  $\gamma$ -CA like domain and are believed to be catalytically inactive. PCC7002 CcmM209 contains all amino acid sequences elements known to be required for catalysis, save C194 and C200. The recombinant protein proved to be inactive. We attempted to reconstitute enzyme activity in PCC7002 CcmM209 by making site directed mutations to restore C194 and C200 along with 3 other mutation to restructure  $\alpha$ C. Homology models for the PCC7002 CcmM209 variants were created using Phyre2 and the coordinates from the X-ray crystal structure (3KWC) to the *T. elongatus* BP-1 enzyme as template. Root mean square deviation of the models improved from 2.39 Å over 1384 atoms for the wild-type to 0.91 Å over 1431 atoms for the V191G-S192Y-G194C-S200C variant. However, no CA activity has been detected in these variants. This suggest that other, unidentified residues, within the *Synechococcus* PCC7002 CcmM209 structure are critical to enzymatic activity despite 78% identity and 90% similarity to its closet active homolog.

[1] Peña, K.L., et al.. *PNAS* 2010. 107: 2455-2460.

### P73: c-di-GMP Signaling in Thermophilic Cyanobacteria

Jun-ya Tamura<sup>1</sup>, Ryouhei Nomura<sup>1</sup>, Toshiyuki Saotome<sup>1</sup>, Yusuke Kawano<sup>1</sup>, Gen Enomoto<sup>1</sup>, Takashi Shimada<sup>2</sup>, Rei Narikawa<sup>1,3</sup>, and Masahiko Ikeuchi<sup>1,4</sup>

<sup>1</sup>Department of Life Sciences (Biology), The University of Tokyo, Tokyo, Japan; <sup>2</sup>Life Science Research Center, Shimadzu Corporation, Tokyo, Japan  
<sup>3</sup>Japan Science and Technology Agency (JST), PRESTO, Saitama, Japan; <sup>4</sup>Japan Science and Technology Agency (JST), CREST, Saitama, Japan

Light can be utilized as energy to drive photosynthetic reactions and also be recognized as critical signal in phototrophic organisms. We have been studying cell aggregation of a thermophilic cyanobacterium *Thermosynechococcus vulcanus* strain RKN that is induced by light and physiological low temperature [1]. Photoinhibition of photosynthetic activity is mitigated by self-shading due to cell aggregation. We also demonstrated that the aggregation is due to cellulose that is accumulated by a putative cellulose synthase TvTII0007 [2]. The cellulase treatment dispersed the aggregates and disruption of *TvtII0007* in *T. vulcanus* abolished the aggregation response. TvTII0007 protein possesses a PilZ domain that has been shown to mediate signaling of c-di-GMP (bis-(3'-5')-cyclic dimeric guanosine monophosphate). This suggests that c-di-GMP signaling may be involved in the cell aggregation. In the genome of *T. elongatus*, there are 5 genes, which encode GGDEF domain, and 5 genes, which encode GGDEF/EAL domain. The GGDEF domain has potential diguanylate cyclase activity and EAL domain has potential phosphodiesterase activity. One of the GGDEF proteins, Tlr0924, was a typical cyanobacteriochrome that binds phycoviolobin as a chromophore and shows reversible photoconversion between blue-absorbing form and green-absorbing form. Enzyme assays revealed that Tlr0924 possesses diguanylate cyclase activity, which was approximately 38-fold higher under irradiation with blue light than green light, indicative of the blue light sensor. When cells of *T. vulcanus* were grown at 31°C under red light, additional irradiation with blue light induced cell aggregation but irradiation with green light did not induce. Such blue light-induced cell aggregation was abolished in *Tvtlr0924* disruptant. These results indicate that Tlr0924 mediates c-di-GMP signaling to the cell aggregation under blue light.

We further screened phenotype of c-di-GMP signaling by overexpression of another GGDEF protein Tlr1210 in *T. elongatus* and *T. vulcanus*. Tlr1210 protein exhibited strong diguanylate cyclase activity. The overexpression of *tlr1210* in motile *T. vulcanus* suppressed its motility even on the genetic background of *TvtII0007* disruption. The overexpressed strain of *T. vulcanus* showed cell aggregation even at 45°C under growth light, while the aggregation was not observed in the overexpression strain on the genetic background of *TvtII0007* disruption. The overexpressed strain of non-aggregating *T. elongatus* showed enhanced accumulation of exopolysaccharides, which was independent of *TvtII0007*, indicative of unknown exopolysaccharides. Thus, c-di-GMP signaling plays multiple roles in thermophilic cyanobacteria in response to blue light and/or other environmental stresses.

[1] Hirano, A., Kunito, S., Inoue, Y. and Ikeuchi, M. Plant Cell Physiol. 38: s37; [2] Kawano, Y., Saotome, T., Ochiai, Y., Katayama, M., Narikawa, R. and Ikeuchi, M. Plant Cell Physiol. 52: 957-66.

### P74: The Fluorescence Recovery Protein - characterization of the switch-off of the OCP-related photoprotection in *Synechocystis* PCC 6803

Michal Gwizdala<sup>1,2</sup>, Adjélé Wilson<sup>1,2</sup> and Diana Kirilovsky<sup>1,2</sup>

<sup>1</sup>CEA, iBiTec-S, Gif sur Yvette, France <sup>2</sup>CNRS, UMR 8221, 91191 Gif sur Yvette, France

*Synechocystis* PCC 6803, like many other strains of cyanobacteria, has developed a blue light-induced photoprotective mechanism that dissipates the excess absorbed light as a harmless heat at the level of the extramembranal photosynthetic antenna, the phycobilisome. This mechanism is triggered by the activation of the soluble Orange Carotenoid Protein (OCP). The OCP carries a ketocarotenoid and is photoactive. Upon illumination the orange dark form (OCP<sup>o</sup>) is converted into the active red form (OCP<sup>r</sup>). In darkness, the OCP<sup>r</sup> reconverts spontaneously to the OCP<sup>o</sup>. *In vitro* this red-to-orange reversion of OCP is temperature dependent. *In vivo*, another protein, the Fluorescence Recovery Protein is the switch-off of the photoprotective mechanism and mutants lacking this protein are unable to regain full capacity of antenna after high light illumination [1]. *In vitro* experiments demonstrated that the FRP interacts with OCP<sup>r</sup> and accelerates its reversion to the OCP<sup>o</sup> [1]. In addition, FRP facilitates the detachment of OCP from the phycobilisome and accelerates the recovery process [2].

In *Synechocystis* PCC 6803 (*Synechocystis*) and in all the other freshwater cyanobacteria strain containing *ocp*-like genes, the gene encoding the FRP (*slr1964*) is directly downstream the *ocp* gene. Comparison of *frp*-like gene sequences from different cyanobacteria strains showed that the FRP of *Synechocystis* (as described in cyanobase) is 25 residues longer than that of all other strains. The residue 26 in *Synechocystis* is a methionine. Studies were realized in our laboratory to elucidate the real size of the FRP in *Synechocystis* cells. *In vivo* and *in vitro* studies using various *Synechocystis* mutants demonstrated that it is the short form of FRP (starting with the Met26) that is present in the *Synechocystis* cells and responsible for the recovery process. The long FRP (starting with the Met 1) is present only when the long-*frp* gene is overexpressed under a strong promoter. Kinetics of the recovery indicated that the long FRP is less active than the short one. We will also describe a point mutation in the FRP N-terminal that decreases the activity of the FRP.

[1] Boulay, C. et al. (2010) PNAS 107, 11620-11625; [2] Gwizdala, M. et al. (2011). The Plant Cell 23, 2631-2643.



## P75: Inactivation of Two Enzymes of the Cyanophycin Degradation Pathway Affects Diazotrophic Growth in the Cyanobacterium *Anabaena* sp. PCC 7120

**Mireia Burnat, Antonia Herrero and Enrique Flores**

Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas and Universidad de Sevilla, E-41092 Sevilla, Spain

*Anabaena* sp. PCC 7120 is a heterocyst-forming filamentous cyanobacterium capable of performing oxygenic photosynthesis in the vegetative cells and nitrogen fixation in differentiated cells called heterocysts [1]. Under unbalanced growth conditions, excess nitrogen is stored in the form of cyanophycin granules, which can be subsequently utilized under N limiting conditions. Cyanophycin is a nonribosomally synthesized peptide composed of multi-L-arginyl-poly-L-aspartate ( $\alpha$ -amino groups of arginine residues linked to  $\beta$ -carboxyl groups of a polyaspartate backbone). Cyanophycin accumulates in the cytoplasm in the form of granules and in the heterocyst at the cellular poles adjacent to the vegetative cells. A cyanophycin-synthesizing enzyme, cyanophycin synthetase, adds both L-aspartic acid and L-arginine to a cyanophycin polymer. During cyanophycin degradation, two enzymes are implicated: cyanophycinase, an exopeptidase, produces  $\beta$ -Asp-Arg dipeptides, and an isoaspartyl-dipeptidase, similar to a plant-type asparaginase, hydrolyses  $\beta$ -Asp-Arg dipeptides [2]. Studies of mutational analysis and gene expression have been carried out with cyanophycin synthetase and cyanophycinase to understand the role of these enzymes *in vivo* [3]. The present study aims to elucidate some questions about the role of cyanophycin as the nitrogenous reservoir for the vegetative cells in the diazotrophic filament of *Anabaena* sp. PCC 7120. We focus on the catabolic metabolism of cyanophycin and the possible role of the arginine produced from hydrolysis of the isoaspartyl-dipeptide as a source of nitrogen in the diazotrophic filament of the cyanobacterium. Two ORFs, *all3922*, encoding an isoaspartyl-dipeptidase [2], and *alr2310*, encoding an enzyme similar to agmatinase [4], have been investigated through the generation of mutants that bear those genes inactivated. Growth tests in liquid media showed that both mutants had a growth rate slightly lower than the wild-type strain in media supplemented with ammonium or nitrate. In contrast, in medium with no combined nitrogen, the  $\Delta$ *all3922* mutant showed a decreased growth rate (70% that of the wild type) and  $\Delta$ *alr2310* mutant did not grow. Filaments of the mutants from cultures supplied with ammonium and nitrate showed extensive granulation in the cytoplasm compared to the wild type. Cells from the  $\Delta$ *all3922* mutant showed an altered and swollen morphology when growing in N-free medium. Studies of arginine catabolism confirmed that Alr2310 is an agmatinase. Our results suggest a role in cyanophycin catabolism of the products of both genes, being Alr2310 essential for the microorganism when growing under diazotrophic conditions.

[1] Flores E. & Herrero A. (2010) *Nat Rev Microbiol* 8:39-50; [2] Hejazi M. et al. (2002) *Biochem J* 364:129-136; [3] Picossi S. et al. (2004) *J Biol Chem* 279:11582-11592; [4] Kaneko T. et al. (2001) *DNA Res* 8:205-213

## P76: RpaB is involved in transcriptional regulation in response to the circadian clock as well as high light stress in *Synechococcus elongatus* PCC 7942

**Mitsumasa Hanaoka<sup>1,2,3</sup>, Naoki Takai<sup>3,4,5</sup>, Norimune Hosokawa<sup>6</sup>, Masayuki Fujiwara<sup>7</sup>, Yuki Akimoto<sup>1,2</sup>, Nami Kobori<sup>1</sup>, Hideo Iwasaki<sup>6,8</sup>, Takao Kondo<sup>3,4</sup>, and Kan Tanaka<sup>1,2,3,9</sup>**

<sup>1</sup>Graduate School of Horticulture, Chiba University, Chiba, Japan; <sup>2</sup>Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan. <sup>3</sup>Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan; <sup>4</sup>Graduate School of Science, Nagoya University, Nagoya, Japan; <sup>5</sup>Graduate School of Nanobioscience, Yokohama City University, Yokohama, Japan; <sup>6</sup>Department of Electrical Engineering and Biological Science and Center for Advanced Biomedical Sciences (TWINS), Waseda University, Tokyo, Japan; <sup>7</sup>Graduate School of Advanced Integration Science, Chiba University, Chiba, Japan; <sup>8</sup>Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Tokyo, Japan; <sup>9</sup>Chemical Resources Laboratory, Tokyo Institute of Technology, Yokohama, Japan

The circadian clock of cyanobacteria is composed of KaiA, KaiB, and KaiC proteins, and the SasA-RpaA two-component system has been implicated in the regulation of one of the output pathways of the clock. We previously demonstrated that a response regulator that is essential for viability, the RpaA homolog, RpaB, is required for transcriptional regulation of high light stress-dependent genes [1]. We showed that RpaB could function as a repressor under the normal light conditions and that high light conditions result in the release of the repression [2]. In this study, we showed that RpaB similarly plays a central role in the transcriptional oscillation of clock-regulated genes [3]. *In vivo* and *in vitro* analyses revealed that RpaB and not RpaA could specifically bind to the *kaiBC* promoter, possibly repressing transcription during subjective night. This suggested that binding may be terminated by RpaA to activate gene transcription during subjective day. Moreover, we found that *rpoD6* and *sigF2*, which encode group-2 and group-3 sigma factors for RNA polymerase, respectively, were also targets of the RpaAB system, suggesting that a specific group of sigma factors can propagate genome-wide transcriptional oscillation. These results thus suggest that RpaB is involved in transcriptional regulation in both high light stress response and the circadian output pathway.

[1] Seki A., Hanaoka M., Akimoto Y., Masuda S., Iwasaki H. and Tanaka K. (2007) Induction of a group 2 sigma factor, RPOD3, by high light and the underlying mechanism in *Synechococcus elongatus* PCC 7942. *J Biol Chem.* 282 (51), 36887-36894; [2] Hanaoka M. and Tanaka K. (2008) Dynamics of RpaB-promoter interaction during high light stress, revealed by chromatin immunoprecipitation (ChIP) analysis in *Synechococcus elongatus* PCC 7942. *Plant J.* 56 (2), 327-335; [3] Hanaoka M., Takai N., Hosokawa N., Fujiwara M., Akimoto Y., Kobori N., Iwasaki H., Kondo T. and Tanaka K. (2012) RpaB, another response regulator operating circadian clock-dependent transcriptional regulation in *Synechococcus elongatus* PCC 7942. *J Biol Chem.* (in press)



## P77: SyR1 - a sRNA regulating photosynthesis in cyanobacteria

Nils Schürgers<sup>1</sup>, Ekaterina Kuchmina<sup>1</sup>, Dennis Dienst<sup>2</sup>, Jens Georg<sup>3</sup>, Wolfgang R. Hess<sup>3</sup>, Annegret Wilde<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Germany; <sup>2</sup>Institute of Biology, Humboldt-University Berlin, Germany; <sup>3</sup>Faculty of Biology, University Freiburg, Germany

Post-transcriptional gene regulation by *trans* encoded small RNAs (sRNAs) is emerging as a common regulatory feature in most prokaryotes. Recently, biocomputational prediction [1], comparative transcriptional analysis [2] and *high throughput pyrosequencing* of *Synechocystis* sp. PCC6803 total RNA [3] revealed many new putative sRNAs in this cyanobacterial model organism. One of these candidates is *Synechocystis* ncRNA 1 (SyR1), an abundant 130nt transcript from the intergenic region between the *fabX* and *hoxH* genes. More detailed investigation of SyR1 showed that it is upregulated under high-light stress and CO<sub>2</sub> depletion [2]. In addition, a SyR1 overexpressing strain exhibits a bleaching-phenotype as it lacks photosynthetic pigments. A homology search revealed putative SyR1 homologues 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 several photosynthesis genes. Moreover, gel mobility shift assays provide evidence for a direct interaction between SyR1 and *psaL*. Ongoing mutational analysis of the putative SyR1 binding site aims to verify the post-transcriptional regulation of this target gene. 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% CO<sub>2</sub> quickly abolishes SyR1 accumulation in the overexpression strain and rescues the bleaching-phenotype. Taken together, we speculate that SyR1-dependent gene regulation affects photosystem biosynthesis and homeostasis, and possibly integrates light and Ci-signaling pathways.

[1] Voss B, Georg J, Schön V, Ude S, Hess WR (2009) Biocomputational prediction of non-coding RNAs in model cyanobacteria. *BMC Genomics* 10:123; [2] Georg J, Voss B, Scholz I, Mitschke J, Wilde A, Hess WR (2009) Evidence for a major role of antisense RNAs in cyanobacterial gene regulation. *Mol Syst Biol* 5:305; [3] Mitschke J, Georg J, Scholz I, Sharma CM, Dienst D, Bantscheff J, Voß B, Steglich C, Wilde A, Vogel J, Hess WR (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC6803. *PNAS* 108(5):2124-9

## P78: Beyond 2-OG sensing: energy charge fundament of the cyanobacterial P<sub>II</sub> signaling

Oleksandra Fokina and Karl Forchhammer

Interfaculty Institute of Microbiology and Infection Medicine, Eberhard-Karls-Universität Tübingen, Tübingen, Germany

P<sub>II</sub> 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<sub>II</sub> 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<sub>II</sub> 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<sub>II</sub> signaling, including perception of 2-OG as C/N balance signal, as well as directly effecting P<sub>II</sub>-receptor interactions. Strikingly, energy charge affects P<sub>II</sub> interaction with the targets NAGK and PipX in different ways: whereas increasing ADP attenuates the interaction with NAGK, it favors the binding to PipX. Thus, low energy charge (high ADP) tunes down the P<sub>II</sub>-mediated activation of arginine biosynthesis at low 2-OG levels (corresponding to nitrogen-excess conditions). On the other hand it enhances the interaction of P<sub>II</sub> with PipX and overrides the effect of 2-OG, thereby preventing PipX-mediated activation of transcription factor NtcA under high 2-OG (nitrogen-poor) conditions. New structures of P<sub>II</sub> in complex with signaling molecules explain the fundamentals of target-specific energy charge and 2-OG sensing.

[1] Fokina O., Chellamuthu VR., Zeth K., & Forchhammer K. (2010) A Novel Signal Transduction Protein PII Variant from *Synechococcus elongatus* PCC 7942 Indicates a Two-Step Process for NAGK-PII Complex Formation. *J. Mol. Biol.* 399:410-421; [2] Fokina O., Herrmann C. & Forchhammer K. (2011) Signal transduction protein PII from *Synechococcus elongatus* PCC 7942 senses low adenylate energy charge *in vitro*. *Biochem. J.* 440:147

## P79: RNA processing in cyanobacteria

**Olga Siadat<sup>1</sup>, Thomas Wallner<sup>1</sup>, Nils Schürgers<sup>1</sup> and Annegret Wilde<sup>1</sup>**

<sup>1</sup>Institute of Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Giessen, Germany

Degradation of RNA is an important element in the control of gene expression in all domains of life. The RNA turnover is relatively well studied in *Escherichia coli* where it is mostly initiated with the endoribonucleolytic cleavage mainly mediated by RNase E. RNase E in *E. coli* cleaves single-stranded RNA with the preference of AU-rich regions. It is also a major element in a multienzyme complex known as the RNA degradosome.

In cyanobacteria RNA processing and degradation is most likely mediated by RNase E/G, RNase J and RNase III homologs. *Synechocystis* sp. PCC 6803 harbours an RNase E/G that is very similar to RNase E and RNase G of *E. coli* in its N-terminal region. However, cyanobacterial RNase E/G is lacking C-terminal scaffold domain that is necessary for the assembly of the degradosome in *E. coli*. Degradosome structures have not so far been identified in cyanobacteria. Nevertheless, RNase E/G is essential for *Synechocystis* sp. PCC 6803 as the disruption of its gene is lethal for the cells. RNase III specifically degrades double-stranded RNA. In comparison to other bacteria *Synechocystis* sp. PCC 6803 possesses 2 copies of the RNase III gene. Simultaneous disruption of both copies of the RNase III gene is lethal for the organism suggesting high biological importance of this endoribonuclease. RNase J is best characterized in *Bacillus subtilis*. In this model organism it has been identified as the first bacterial enzyme possessing both endo- and 5'-to-3' exoribonucleolytic activity. However, the functional properties of RNase J homolog in *Synechocystis* sp. PCC 6803 are not clearly understood. It has only been shown that RNase J is essential for the organism as it was not possible to construct a knockout mutant.

In order to elucidate specific functions of RNases in cyanobacteria we constructed overexpression mutants with FLAG-tagged RNase E/G, RNase J and two RNase III homologs in *Synechocystis* sp. PCC 6803. We are currently performing *in vitro* cleavage assays with these purified recombinant proteins. RNase E/G seems to be responsible for processing of some Hfq-dependent noncoding RNAs that have single-stranded AU-rich structures. The ability of RNase III to specifically degrade double-stranded RNA complexes makes it an ideal candidate for processing antisense RNAs base paired with their targets. With the help of purified recombinant RNases from *Synechocystis* sp. PCC 6803 we are also planning to conduct co-immunoprecipitation experiments to investigate potential RNA degrading complexes.

## P80: The iron-sulfur centers of *Nostoc punctiforme* HupS

**Patrícia Raleiras<sup>1</sup>, Petra Kellers<sup>2</sup>, Ann Magnuson<sup>1</sup>, Peter Lindblad<sup>1</sup>, and Stenbjörn Styring<sup>1</sup>**

<sup>1</sup>Photochemistry and Molecular Science, Department of Chemistry – Ångström Laboratory, Box 523, Uppsala University, SE-75120 Uppsala, Sweden; patricia.raleiras@kemi.uu.se; <sup>2</sup>Department of Theoretical and Computational Biophysics, Max Planck Institute for Biophysical Chemistry, Am Faßberg 11, D-37077 Göttingen, Germany

The uptake hydrogenase HupSL is the only hydrogenase present in the filamentous cyanobacterium *Nostoc punctiforme* ATCC 29133. It is expressed exclusively in heterocysts, where it oxidizes H<sub>2</sub> produced by nitrogenase under N<sub>2</sub>-fixing conditions[1][2]. HupSL is composed of the large subunit HupL, harbouring the nickel-iron active site, and the small subunit HupS, containing three electron-transfer iron-sulfur (FeS) clusters: a proximal [4Fe-4S] (closer to the active site on HupL), a medial [3Fe-4S] and a distal [4Fe-4S] (closer to the enzyme's surface). Cyanobacterial HupS possess unique FeS cluster binding motifs on both [4Fe-4S] clusters: a CXXN motif in the proximal cluster, and a QXXC motif in the distal one[3][4]. Most NiFe hydrogenases characterized to date have been isolated from non-phototrophic bacteria, whereas their cyanobacterial counterparts are poorly understood.

To study the redox properties of cyanobacterial HupS, this subunit was for the first time heterologously expressed and purified without the nickel-containing subunit. This allows independent characterization of the FeS clusters without interference from a magnetic or redox coupling to the active site. *N. punctiforme* hupS was cloned in the vector pET43.1a(+) (Novagen) to produce a fusion construct with the solubilization protein Nus•Tag™. A Strep(II)-tag was included in HupS C-terminal. The resulting NusAHupS fusion protein was expressed in *E. coli* BL21(DE3) and purified in both aerobic and anaerobic conditions. NusAHupS exhibited a UV-visible absorption spectrum typical of FeS proteins, with broad metal-to-ligand charge transfer bands around 330 and 420nm. Low temperature electron paramagnetic resonance spectroscopy of NusAHupS showed that the FeS centers undergo oxidative degradation upon exposure to atmospheric O<sub>2</sub>; also, a high-spin species previously undetected in other hydrogenases was found in the anaerobically purified protein, and attributed to a reduced state of [4Fe-4S] clusters. Results are discussed in the context of the nitrogen fixation-related hydrogen metabolism of the heterocyst.

[1] Tamagnini, P.; Troshina, O.; Oxelfelt, F.; Salema, R.; Lindblad, P., Appl Environ Microbiol 63 (1997) 1801–1807; [2] Camsund, D.; Devine, E.; Homqvist, M.; Yohanoun, P.; Lindblad, P.; Stensjö, K., FEMS Microbiol Lett 316 (2011) 152–159; [3] Oxelfelt, F.; Tamagnini, P.; Lindblad, P., Arch Microbiol 169 (1998) 267–274; [4] Schröder, O.; Bleijlevens, B.; de Jongh, T. E.; Chen, Z.; Li, T.; Fischer, J.; Förster, J.; Friedrich, C. G.; Bagley, K. A.; Albracht, S. P. J.; Lubitz, W., J Biol Inorg Chem 12 (2007) 212–233

### P81: Hydrogen(ases) and nitrogenase activity of two halotolerant purple non-sulfur isolates

**Amal W. Danial, A.M.Abdel Wahab and R. Abdel-Basset**

Botany Department, Faculty of Science, Assiut University, 71516Assiut, Egypt

Photoproduction of hydrogen, nitrogenase and hydrogenase activity were studied in two purple non-sulfur bacteria, isolated from saline water ditches close to the sea shore in the northern west coast of Alexandria city. These two isolates were representing the lowest (F) and highest (D) salinity tolerance. Highest growth rate, nitrogenase and hydrogenase activity of isolate D (pink) and F (brown) have been displayed on the 6<sup>th</sup> day. The highest yield of H<sub>2</sub> production accounted to 550 and 370 ml/culture of D and F; respectively. Acetate was the most suitable carbon source compared with lactate or succinate in the sense of highest rate of the studied parameters. In both strains, the optimum concentration of acetate, lactate and succinate were 22mM, 50mM and 50mM; respectively. A 50% decrease in the optimum acetate concentration promptly dropped hydrogen yield down to 12.6%. Nitrate (10mM) induced the highest rate of growth and enzymes activity compared with equimolar concentrations of glutamate and ammonia. Glycine betaine (10 mM) and alginate 2% significantly enhanced hydrogen evolution up to 6 and 8 fold; respectively. Their combination further increased the previous parameters to 12 folds. Hydrogenase-dependent hydrogen evolution accounted only to 6.4 and 4.4% relative to nitrogenase proportion in D and F cultures; respectively. Full identification of D and F is underway.

### P82: Creation of chimera photoreceptors to develop various light switches for effective photosynthesis-based bioenergy production

**Rei Narikawa<sup>1,2</sup>, Ni Ni Win<sup>1</sup> and Masahiko Ikeuchi<sup>1,3</sup>**

<sup>1</sup>Department of Life Sciences, University of Tokyo, Tokyo, Japan; <sup>2</sup>PRESTO, Japan Science and Technology Agency, Saitama, Japan; <sup>3</sup>CREST, Japan Science and Technology Agency, Saitama, Japan;

In order to effectively perform photosynthetic reaction, photosynthetic organisms acclimate to changing light environments by using various photoreceptors via transcriptional and enzymatic regulations. In fact, cyanobacteria possess many photoreceptors and show many photoresponses such as phototaxis, complementary chromatic acclimation and light-induced cell aggregation by using specific photoreceptors [1]. Especially, cyanobacteriochromes, which are found only in cyanobacteria and similar to but clearly distinct from the red/far-red light reversible phytochromes, show various spectral properties that cover throughout the near UV/vis region. Further, these photoreceptors possess various output enzymatic domains such as histidine kinase, c-di-GMP synthase and c-di-GMP phosphodiesterase. In this study, we created many chimera proteins of novel combination of photosensory and enzymatic domains to develop various light switches. We tested the enzymatic activities of these chimera proteins in both forms to clarify which form is active one. We will apply these light switches to photosynthesis-based bioenergy production that is recently significant issue, but many improvements in the production efficiency are needed for practical utilization. We will present a strategy to construct dark/light switch and growth/production switch to establish effective production of extracellular polysaccharides such as cellulose.

[1] Ikeuchi M & Ishizuka T (2008) Cyanobacteriochromes: a new superfamily of tetrapyrrole-binding photoreceptors in cyanobacteria. *Photochem. Photobiol. Sci.* 7: 1159-1167.

### P83: Analysis of a transcriptional regulator ChIR that activates the expression of tetrapyrrole biosynthesis genes in response to low-oxygen conditions

**Rina Aoki<sup>1</sup>, Tomoya Takeda<sup>1</sup>, Tatsuo Omata<sup>1</sup>, Kunio Ihara<sup>2</sup>, and Yuichi Fujita<sup>1</sup>**

<sup>1</sup>Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; <sup>2</sup>Center for Gene Research, Nagoya University, Nagoya, Japan

Tetrapyrroles including heme, chlorophyll and bilins are essential molecules for photosynthetic organisms. Their biosynthetic pathway consists of more than twenty enzymatic reactions. Among them, three reactions, coproporphyrinogen III oxidase (HemF), Mg-protoporphyrin IX monomethyl ester cyclase (ChIA<sub>I</sub>) and heme oxygenase (HO1) use oxygen molecules as the substrate. Therefore, these enzyme activities would be reduced under low-oxygen conditions. In the cyanobacterium *Synechocystis* sp. PCC 6803, these oxygen-dependent reactions are bypassed by 'low-oxygen' type enzymes (HemN, ChIAII and HO2) to adapt to low-oxygen conditions [1, 2, 3]. Genes encoding these enzymes (*hemN*, *chIA<sub>II</sub>* and *ho2*) form an operon (*chIA<sub>II</sub>-ho2-hemN*) and its expression is induced under low-oxygen conditions. Recently, we identified a transcriptional regulator involved in the induction of the operon and proposed to name it 'ChIR' [4]. ChIR is a MarR family transcriptional regulator conserved in a wide variety of prokaryotes. In the *chIR*-lacking mutant  $\Delta chIR$ , the expression of the genes encoding the low-oxygen type enzymes was suppressed under low-oxygen conditions.  $\Delta chIR$  showed severe growth retardation and decrease in chlorophyll contents under low-oxygen conditions because of the defect of the low-oxygen type enzymes. From these results, it is suggested that ChIR acts as a transcriptional activator under low-oxygen conditions. In our working model, while ChIR is maintained as an inactive form not to bind to the promoter under aerobic conditions and its target genes are not expressed, ChIR is converted to an active form to bind to the promoter to activate the transcription of the target genes upon the exposure to low-oxygen conditions. We also isolated a ChIR-D35H variant functioning as a superactivator. Gel mobility shift assay indicated that ChIR-D35H binds to the promoter region of *chIA<sub>II</sub>* even under aerobic conditions. This result suggested that ChIR-D35H is fixed as an active form. However, it remains unknown how ChIR converts between the active and inactive forms in response to the environmental oxygen levels. To dissect the switching mechanism, analysis of amino acid substitution variants of ChIR is in progress.

[1] Goto, T.; Aoki, R.; Minamizaki, K.; Fujita, Y. *Plant Cell Physiol.* 2010, 51, 650; [2] Minamizaki, K.; Mizoguchi, T.; Goto, T.; Tamiaki, H.; Fujita, Y. *J. Biol. Chem.* 2008, 283, 2684; [3] Aoki, R.; Goto, T.; Fujita, Y. *Plant Cell Physiol.* 2011, 52, 1744; [4] Aoki, R.; Takeda, T.; Omata, T.; Ihara, K.; Fujita, Y. *J. Biol. Chem.* 2012, 287, 13500.

## P84: A 50-kb nitrogen fixation gene cluster contains the transcriptional regulator gene *chlR* for chlorophyll biosynthesis genes under anaerobic conditions in the cyanobacterium *Leptolyngbya boryana*

Ryoma Tsujimoto<sup>1</sup> and Yuichi Fujita<sup>1</sup>

<sup>1</sup>Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

Nitrogen is an essential nutrient for all organisms. Some cyanobacteria utilize N<sub>2</sub> as the sole nitrogen source by nitrogen fixation catalyzed by nitrogenase. Nitrogenase is a metalloenzyme extremely vulnerable to oxygen. It remains to be solved how nitrogenase functions in oxygenic photosynthetic organisms while circadian regulation appears to be one of the major regulations. Here we report a large gene cluster for nitrogen fixation in the non-heterocystous filamentous cyanobacterium *Leptolyngbya boryana*. We determined nucleotide sequence of 49,462 bp in the genome of *L. boryana* starting from the *nifH* gene encoding nitrogenase Fe protein [1] by inverse PCR or PCR using degenerate primers. This region contains 50 ORFs, including *nifD* and *nifK* encoding nitrogenase MoFe protein and genes for nitrogenase metallocluster synthesis. In addition, genes for ferredoxins, cytochrome c oxidase, molybdenum transporter and some enzymes involved in anaerobic fermentation pathway were contained in the cluster. Moreover, a gene encoding the MarR-type transcription factor was found in this cluster. It shows high similarity to *chlR* (*sll1512*) of non-nitrogen fixing cyanobacterium *Synechocystis* sp. PCC 6803 (65% identity). ChlR activates the expression of tetrapyrrole biosynthesis genes under anaerobic conditions [2]. To investigate the function of *L. boryana chlR* (*LbchlR*), a gene knockout mutant K3 was isolated by double homologous recombination. While K3 grew normally under nitrate-supplied and aerobic conditions, it showed poor growth under anaerobic conditions with or without nitrate. Pigment analysis indicated that the K3 cells grown under nitrogen-fixing conditions contained a greatly reduced level of chlorophyll *a* (18% of WT) accompanied with significant accumulation of chlorophyll intermediates; coproporphyrinogen III, protoporphyrin IX, Mg-protoporphyrin IX monomethylester (MPE) and protochlorophyllide. The induction of the *nif* and other genes in the gene cluster under nitrogen-fixation conditions was not affected in K3 cells. In contrast, the operon consisting *chlA<sub>II</sub>* (MPE cyclase), *ho2* (heme oxygenase 2) and *hemN* (coproporphyrinogen III oxidase) located in the other locus in the genome was not induced under anaerobic conditions. These three genes are necessary for tetrapyrrole biosynthesis under low oxygen conditions. The phenotype of K3 are similar to the *chlR*-lacking mutant of *Synechocystis* sp. PCC 6803 [2]. The results suggested that LbChlR activates the tetrapyrrole biosynthesis under anaerobic conditions where nitrogen fixation takes place and it plays an important role to supply Chl supporting the energy demand for nitrogen fixation.

[1] Fujita Y et al. (1991) Plant Cell Physiol. 32: 1093-1106;

## P85: Redox regulation of Universal Stress Protein Usp1 in *Synechocystis* sp. PCC 6803

Alejandro Mata-Cabana<sup>1,2</sup>, Sandra Díaz-Troya<sup>1</sup>, Francisco J. Florencio<sup>1</sup>, and Anna M. Lindahl<sup>1</sup>

<sup>1</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, Universidad de Sevilla-CSIC, Avda Américo Vespucio 49, 41092-Sevilla, Spain; <sup>2</sup>Present address: Department of Genetics, University Medical Centre Groningen, University of Groningen, PO Box 30001, 9700 RB Groningen, The Netherlands

The Universal Stress Protein (USP) is a superfamily of proteins present in numerous prokaryotic as well as eukaryotic organisms [1]. The precise function of the Usp-domain is not clear, although it is known to possess autophosphorylating activity and it can be found either alone, in tandem or as a fusion with other domains. The UspA protein from *E. coli* initially gave the name to the superfamily since the levels of this protein become elevated in response to a large variety of stress conditions such as nutrient deprivation, heat, oxidants, uncouplers of the electron transport chain or antibiotics [1, 2]. We have identified Usp1 (*slr0244*) and Usp2 (*slr0670*) as membrane associated thioredoxin TrxA targets in *Synechocystis*. They both consist of two Usp-domains in tandem, and have four cysteines in their amino acid sequences. So, to gain insight into the molecular mechanism of Usp proteins, we decided to analyze the redox regulation of Usp1. Under oxidizing conditions Usp1 is mainly in an oligomeric form with a very high molecular mass. TrxA is able to reduce Usp1 to its monomeric form *in vitro* using DTT as electrons donor and this reduction can be reverted with oxidizing agents (Cu<sup>2+</sup>, H<sub>2</sub>O<sub>2</sub>) treatment. Furthermore, we have found that Usp1 is able to autophosphorylation, and this activity is impaired under reducing conditions. In the cells, Usp1 localizes in the membrane fraction, and treatments such as the uncoupler CCCP and high light, induce an increase in Usp1 levels and its oligomerization.

This work has been financed by EU funds (FEDER) grant BFU2010-17508 and Plan E (IEO-USE).

[1] Kvint K., et al. (2003) Curr Opin Microbiol. 6:140-5; [2] Gustavsson N., et al. (2002) Mol Microbiol. 43:107-17.

**P86: Regulation of glycogen metabolism in the nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120****Shigeki Ehira<sup>1,2</sup>, and Masayuki Ohmori<sup>1</sup>**<sup>1</sup>Department of Biological Science, Faculty of Science and Engineering, Chuo University, Tokyo, Japan; <sup>2</sup>PRESTO, Japan Science and Technology Agency, Saitama, Japan

*Anabaena* sp. strain PCC 7120 is a filamentous cyanobacterium, in which certain vegetative cells differentiate into heterocysts that are specialized cells for nitrogen fixation. Heterocysts are unable to carry out photosynthesis and depend on vegetative cells for carbohydrate to generate reducing power required for nitrogen fixation. Thus, carbohydrate metabolism is very important for nitrogen fixation in the filamentous cyanobacteria, however, its regulatory mechanism remains unknown. We have recently found that a nitrogen-regulated response regulator NrrA, which is a transcriptional regulator involved in heterocyst differentiation [1,2], controls glycogen catabolism. The transcript levels of genes involved in glycogen catabolism, such as *glgP1* and *xfp-gap1-pyk1-talB* operon, are decreased by the *nrrA* disruption. Moreover, glycogen accumulation and depression of nitrogenase activities are observed in this disruptant. NrrA binds specifically to the promoter region of *glgP1*, encoding a glycogen phosphorylase, and also to the promoter region of *sigE*, encoding a group 2 sigma factor of RNA polymerase. SigE activates expression of the *xfp* operon, encoding enzymes of glycolysis and the pentose phosphate pathway. It is concluded that NrrA controls not only heterocyst differentiation but also glycogen catabolism in *Anabaena* PCC 7120 [3].

[1] Ehira, S., and M. Ohmori (2006) NrrA, a nitrogen-responsive response regulator facilitates heterocyst development in the cyanobacterium *Anabaena* sp. strain PCC 7120. *Mol. Microbiol.* 59:1692-1703; [2] Ehira, S., and M. Ohmori (2006) NrrA directly regulates expression of *hetR* during heterocyst differentiation in the cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Bacteriol.* 188:8520-8525; [3] Ehira, S., and M. Ohmori (2011) NrrA, a nitrogen-regulated response regulator, controls glycogen catabolism in the nitrogen-fixing cyanobacterium *Anabaena* sp. strain PCC 7120. *J. Biol. Chem.* 286:38109-38114.

**P87: Single-cell Confocal microspectrometry of a filamentous cyanobacterium *Nostoc* in Culture and Symbiosis with Lichen****Shigeru Itoh<sup>1,2</sup>, Kana Sugiura<sup>2</sup>, Keisuke Sato<sup>2</sup>, Masayuki Komura<sup>2</sup>, Hisanori Yamakawa<sup>2</sup> and Ikuko Iwasaki<sup>3</sup>**<sup>1</sup>Center for Gene Research, Nagoya University, Nagoya 464-8602, Japan; <sup>2</sup>Division of Material Science (Physics), Graduate School of Science, Nagoya University; <sup>3</sup>Faculty of Bioresources, Akita Prefectural University, Akita, Japan

Confocal single-cell micro-spectrometry<sup>1,2</sup> was performed to evaluate the pigment composition and differentiation/fluctuation of filamentous cyanobacteria *Nostoc* species in cultured and symbiotic conditions in lichen *Peltigera polydactylon*. Contents of phyco-cyanin (PC), allophycocyanin (APC), and chlorophyll a on photosystems I and II (PSI and PSII) in vegetative (*veg*) and heterocyst (*het*) cells were evaluated based on single-cell fluorescence spectra<sup>1</sup>.

- 1) Fluorescence and absorption spectra of single cells, measured at 298 and 40 K, indicated low contents of PSII and PC in *het* cells.
  - 2) F684/F730 (PSII/PSI) ratios varied widely at 1.1-2.4 in 292 *veg* cells, and were low at 0.7-0.9 for 19 *het* cells.
  - 3) *Veg* cells in a single filament showed a low fluctuation at 7.7 %, suggesting the fast mutual communication between the cells. The fluctuation was fairly smaller than that at 14.5 % among cells in different filaments.
  - 4) Some *veg* cells that had specifically low PSII/PSI or APC/PSII in filaments at the center between two *het* cells, are assigned as pro-heterocysts differentiating into *het* cells.
  - 5) Fluorescence spectra at 40 K indicated the mature PSII bands even in *het* cells.
  - 6) APC/PSII fluorescence ratio was almost constant in all *veg* and most of *het* cells, although PC and PE fluorescence were increased in the latter.
- Single-cell spectrometry was further applied to symbiotic *Nostoc* cells in a lichen *Peltigera polydactylon* and in culture in BG11±N media after isolation.
- 7) Symbiotic *Nostoc* cells showed PSII/PSI ratios different from cultured cells.
  - 8) Symbiotic cells showed high APC/PSII fluorescence ratio comparable to cells cultured in the N-rich medium. The result suggests that symbiotic *Nostoc* cells live in a N-abundant environment inside *P. polydactylon*.
  - 9) APC/PSII fluorescence showed further high ratio depending on localization in lichen, season, location, and species.
  - 10) Almost no *het* cells were found inside two species of *P. polydactylon*.

Single-cell micro-spectrometry at room and cryogenic temperatures is shown to indicate the pigment composition and function of each cell quantitatively. Statistical analysis of the large numbers of obtained spectra allows evaluation of dynamic behaviors of photosynthetic prokaryotes without external probes.

[1] Itoh, S. and Sugiura, K. (2005). Fluorescence in photosystem I. In *Chlorophyll fluorescence: A signature of photosynthesis*, pp 231-250, Kluwer; [2] Shibata, Y., Saga, Y., Tamiaki, H. and Itoh, S. (2006) Low-temperature fluorescence from single chlorosomes of green filamentous and sulfur bacteria. *Biophys. J.* 91: 3787-3796.



## P88: Molecular inheritance of the cyanobacterial circadian clock

Susan E. Cohen<sup>1,2</sup>, Marcella L. Erb<sup>2</sup>, Joseph A. Pogliano<sup>2</sup> and Susan S. Golden<sup>1,2</sup>

<sup>1</sup>Center for Chronobiology, University of California-San Diego, La Jolla, CA 92093-0116; <sup>2</sup>Division of Biological Sciences, University of California-San Diego, La Jolla, CA 92093-0116

The timing of cell division is coordinated with other cyclic events, of other periodicities, in the lives of cells. From bacteria, to algae, to regenerating liver cells of mammals, the circadian biological clock controls the time of day during which cell division can occur<sup>1,2</sup>. The mechanism and function of this time restriction, or gating, of cell division is poorly understood in any system. The circadian control of cell division in cyanobacteria provides an opportunity to assess both how and why these processes are interlocked. The model organism *Synechococcus elongatus* is a unicellular cyanobacterium for which genetic manipulation is simple, circadian rhythms of gene expression are readily measured, and extensive genetic tools are available. In *S. elongatus* the number of genomes per cell in culture as well as the superhelical status and compaction of the chromosomes, oscillate with circadian periodicity<sup>1, 3-5</sup>. Furthermore, the topological status of the chromosome is highly correlated with a distinct state in gene expression, and has been proposed to be a key factor in imparting circadian gene expression patterns<sup>3</sup>. Although *S. elongatus* can divide once or more during a single circadian cycle, the fidelity of the clock is remarkably stable and inherited with perfect phase from mother to daughter cell<sup>6</sup>. This project aims to resolve the subcellular localization of chromosomes and fluorescently tagged oscillator proteins during the cell and circadian cycles. How clock proteins and chromosomes are inherited and related to the gating of cell division will be investigated. Together, these data will enlighten our understanding of the relationship between the cell and circadian cycles within the three-dimensional architecture of intact cells.

[1] Mori T, Binder B, Johnson CH. Circadian gating of cell division in cyanobacteria growing with average doubling times of less than 24 hours. Proc Natl Acad Sci U S A 1996; 93:10183-8; [2] Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division *in vivo*. Science 2003; 302:255-9; [3] Vijayan V, Zuzow R, O'Shea EK. Oscillations in supercoiling drive circadian gene expression in cyanobacteria. Proc Natl Acad Sci U S A 2009. [4] Smith RM, Williams SB. Circadian rhythms in gene transcription imparted by chromosome compaction in the cyanobacterium *Synechococcus elongatus*. Proc Natl Acad Sci U S A 2006; 103:8564-9; [5] Woelfle MA, Xu Y, Qin X, Johnson CH. Circadian rhythms of superhelical status of DNA in cyanobacteria. Proc Natl Acad Sci U S A 2007; 104:18819-24; [6] Mihalcescu I, Hsing W, Leibler S. Resilient circadian oscillator revealed in individual cyanobacteria. Nature 2004; 430:81-5.

## P89: The SigB $\sigma$ factor up-regulates many high salt and high light protective mechanisms in the cyanobacterium *Synechocystis* sp. PCC 6803

Kaisa Haktila, Liisa Gunnelius, Hanna-Leena Nikkinen, Tuomas Huokko, Taras Antal, Maija Pollari, Esa Tyystjärvi, and Taina Tyystjärvi

Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland

The initiation of transcription, mediated by the RNA polymerase holoenzyme, is the main determinant of gene regulation in eubacteria. The RNA polymerase holoenzyme is composed of a core capable of the polymerization reaction and a  $\sigma$  factor that is responsible for promoter recognition and required for transcription initiation. In *Synechocystis* sp. PCC 6803 the *sigA* gene encodes a primary  $\sigma$  factor essential for cell viability. The group 2  $\sigma$  factors, SigB, SigC, SigD and SigE show extensive sequence and structural similarity with SigA but are non-essential[1], and the SigF-SigI genes encode for alternative  $\sigma$  factors.

Our data shows that *Synechocystis* mutants with one, two or three group 2  $\sigma$  factors inactivated simultaneously in all possible combinations[1,2] and also the  $\Delta$ sigBCDE strain without any group 2  $\sigma$  factor grew well under standard conditions. The SigB factor was found to be important for the growth of *Synechocystis* cells in high salt (0.7 M NaCl) stress[2,3]. Many salt acclimation processes turn out to be up-regulated by the SigB factor[3]. The HspA heat shock protein is one of the most strongly up-regulated proteins in high salt conditions, and HspA was less abundant and more abundant in the  $\Delta$ sigB and  $\Delta$ sigCDE strains, respectively, than in the control strain in high salt. The amount of glucosylglycerol-phosphate synthase, a key enzyme in the production of the compatible solute glucosylglycerol, was lower in the  $\Delta$ sigB strain than in the control strain. Addition of the compatible solute trehalose restored the growth of the  $\Delta$ sigB strain in high salt conditions. Protective carotenoid pigments, especially zeaxanthin and myxoxanthophyll, were up-regulated in the  $\Delta$ sigCDE strain and down-regulated in the  $\Delta$ sigB strain under standard conditions. These same carotenoids were up-regulated in high salt conditions.

The  $\Delta$ sigCDE strain lost light saturated PSII activity more slowly in bright light than the control strain because light-induced damage of PSII, measured in the presence of translation inhibitor lincomycin, occurred more slowly in the  $\Delta$ sigCDE strain. Analysis of possible photoprotective mechanisms in  $\Delta$ sigCDE showed indicated that high amounts of zeaxanthin and myxoxanthophyll provide protection against singlet oxygen, and non-photochemical quenching functioned more efficiently than in the control strain. Furthermore, the photoprotective *flv4-sll2018-flv2* operon was up-regulated in the  $\Delta$ sigCDE strain.

[1] Pollari M, Gunnelius L, Tuominen I, Ruotsalainen V, Tyystjärvi E, Salminen T, Tyystjärvi T (2008) Plant Physiology 147: 1994-2005; [2] Pollari M, Rantamäki S, Huokko T, Kärnlund-Marttila A, Virjamo V, Tyystjärvi E, Tyystjärvi T (2011) Journal of Bacteriology 193: 265-273; [3] Nikkinen H-L, Haktila K, Gunnelius L, Huokko T, Pollari M, Tyystjärvi T (2012) Plant Physiology 158: 514-523.



## P90: Elucidation of mechanisms of transcriptional regulation dependent on thioredoxin in the cyanobacterium *Synechocystis* sp. PCC 6803

Taro Kadowaki<sup>1</sup>, Toru Hisabori<sup>2</sup>, Yukako Hihara<sup>1</sup>

<sup>1</sup>Graduate School of Science and Engineering, Saitama University, Saitama, Japan; <sup>2</sup>Chemical Resources Laboratory, Tokyo Institute of Technology, Kanagawa, Japan

Photosynthetic organisms undergo dynamic rearrangements of components of the photosynthetic apparatus to optimize light capture under low-light (LL) conditions and mitigate the potentially harmful consequences of high-light (HL) conditions, a process known as photosynthetic acclimation. A dramatic change in gene expression profile occurs immediately after the shift from LL to HL conditions and this change is closely related to the subsequent acclimation responses. Transcription of many HL-responsive genes is dependent on the activity of photosynthetic electron transport [1]. We have been characterizing transcriptional regulator working for HL acclimation in the cyanobacterium *Synechocystis* sp. PCC 6803, and identified a small LuxR-type transcriptional regulator PedR working on the regulation dependent on photosynthetic electron transport activity [2].

PedR is working for positive or negative regulation of its target genes under LL conditions. Upon the shift to HL conditions, it becomes transiently inactivated with a concomitant conformational change. We isolated thioredoxin as PedR-interacting factor by pull-down analysis [3]. The conformational change and inactivation of PedR upon the shift to HL were not observed in the mutants deficient in thioredoxin reduction systems, indicating that increased availability of reducing equivalents at the acceptor side of photosystem I inactivates PedR through its reduction via thioredoxin. PedR/thioredoxin system seems to be one of the key components that couple the activity of photosynthetic electron transport and transcriptional regulation required for acclimation responses. However, the number of PedR regulon genes is quite limited and regulatory mechanism of most genes whose expression is dependent on the photosynthetic activity has remained unsolved. We assumed existence of other transcriptional regulators interacting with thioredoxin and established a screening system to detect their interaction. In this system, S-tagged C35S thioredoxin and a His-tagged transcriptional regulator are co-expressed within *E. coli* cells and their interaction can be detected by western-blot analysis. As a positive control experiment, interaction between thioredoxin and PedR was successfully detected. When each of three cysteine residues in PedR, C73, C79 and C80, was substituted to serine, resulted PedR mutants showed lowered affinity to thioredoxin. C80S PedR showed the lowest affinity among them, indicating that C80 of PedR is the target of thioredoxin.

[1] Muramatsu and Hihara (2012) J Plant Res 125: 11-39; [2] Nakamura and Hihara (2006) J Biol Chem 281: 36758-36766; [3] Horiuchi et al. (2010) Biochem J 431: 135-140

## P91: Gene expression indicates a zone of heterocyst differentiation within the thallus of the cyanolichen *Pseudocyphellaria crocata*

Jocelyn P.S. Chua, Emma J.S. Wallace, Jessica A. Yardley and Tina C. Summerfield

Department of Botany, University of Otago, PO Box 56, Dunedin, New Zealand

*Nostoc* strains are found in symbiotic association with fungi and a wide range of plants. In their association with plants, cyanobacteria show increasing morphological and physiological changes as the symbiotic tissue matures [1]. The structure of bipartite cyanolichens enables examination of the symbiotic relationship as it ages – from the younger, growing margins to the older thallus center. We investigated development of the symbiotic association in the bipartite lichen *Pseudocyphellaria crocata* by characterizing the *Nostoc* cyanobiont in two regions of the thallus. A HIP1-based differential display technique was modified to examine gene expression in *Nostoc* strains, including comparing the margin and center of the lichen thallus. Northern hybridization and qRT-PCR were used to confirm differential display results, and to determine expression levels of key cyanobacterial genes. The differential display technique identified differentially expressed genes in the lichenized *Nostoc* strain in the margin compared to the center of the thallus. The expression of genes encoding subunits of cytochrome c oxidase and ATP synthase was increased in the thallus margin compared to the center. The *cox2* operon is heterocyst specific and expression of other heterocyst-specific genes (*hetR* and *nifK*) was also elevated in the margin; whereas, expression of the Photosystem II-specific *psbB* gene as well as the quantum yield of Photosystem II were not elevated in the margins. Data indicate a similar level of photosynthetic activity in the thallus center and margins and an increase in heterocyst differentiation in the margins.

[1] Rai AN, Soderback E, Bergman B. 2000. Cyanobacterium-plant symbioses. New Phytologist 147: 449-481

## P92: The C-terminal domain of BchB subunit is essential for the maximal activity of dark-operative protochlorophyllide reductase from *Rhodobacter capsulatus*

Aya Tatematsu<sup>1</sup>, Jiro Nomata<sup>1</sup>, Norifumi Muraki<sup>2</sup>, Genji Kurisu<sup>2</sup> and Yuichi Fujita<sup>1</sup>

<sup>1</sup>Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan; <sup>2</sup>Institute for Protein Research, Osaka University, Suita, Japan

Dark-operative protochlorophyllide (Pchl<sub>id</sub>) oxidoreductase (DPOR) catalyzes the reduction of D-ring double bond (C17=C18) of Pchl<sub>id</sub> to form chlorophyllide *a* at the final stage of (bacterio)chlorophyll biosynthesis. DPOR consists of two components, L-protein (a BchL dimer) and NB-protein (a BchN-BchB heterotetramer), which are structurally closely related to Fe protein and MoFe protein of nitrogenase, respectively [1]. L-protein plays an ATP-dependent electron donor for NB-protein with a [4Fe-4S] cluster that is extremely sensitive to oxygen [2]. NB-protein provides the catalytic sites carrying a pair of [4Fe-4S] cluster that mediates the electron transfer from the [4Fe-4S] cluster of L-protein to the substrate Pchl<sub>id</sub> [3]. In previous study, we determined the X-ray crystal structures of Pchl<sub>id</sub>-bound and Pchl<sub>id</sub>-free forms of NB-protein from *Rhodobacter capsulatus* and revealed a common architecture to reduce stable multi-bonds such as porphyrin and nitrogen [4]. In addition, we proposed a unique reaction mechanism of the stereo-specific reduction of C17=C18 double bond of Pchl<sub>id</sub>. In this model, Asp274 of BchB and the propionate at C13 of Pchl<sub>id</sub>, the substrate itself, serve as proton donors for C17 and C18 carbons of Pchl<sub>id</sub>, respectively. However, the C-terminal domain of BchB (from Glu420 to the C-terminal Arg525) was disordered in the crystal structures of both forms [4]. Since the amino acid sequence of C-terminal domain is conserved well among all BchB proteins [1], this domain may play some unknown important role in the catalysis. Here we report the crystal structure of the C-terminal domain of BchB and biochemical analysis of NB-protein variants with truncated BchB subunits. The C-terminal domain of BchB (from Arg475 to Tyr523) consists of three short  $\alpha$ -helices and the loops connecting the helices interact with BchN' of the other catalytic unit. To analyze function of the C-terminal domain, a series of truncated and site-directed variants of NB-protein were prepared and their activities were examined. A shortest BchB without C-terminal 115 amino acid residues,  $\Delta$ C115, totally lost DPOR activity while it forms the complex with BchN. The other truncated BchB ( $\Delta$ C85,  $\Delta$ C50 and  $\Delta$ C22) showed about 40 % activity of the wild type. A site-directed variant with the clustered positive charged amino acid residues (Arg502, Lys503, Lys506 and Arg507) substituted with Gln exhibited only 20 % activity. It was suggested that the C-terminal domain is essential for the maximal activity of DPOR.

[1] Fujita Y and Bauer CE (2003) In Porphyrin Handbook, Edited by Kadish, K. M., Smith, K. M., Guillard, R., Vol. 13, pp. 109-156, Academic Press, Oxford, UK; [2] Nomata J et al. (2006) FEBS Lett. 580: 6151-6154; [3] Nomata J et al. (2008) FEBS Lett. 582: 1346-1350; [4] Muraki N et al. (2010) Nature 465, 110-114.

## P93: Involvement of the transcriptional regulator CyAbrB in the central carbon metabolism in *Synechocystis* sp. PCC 6803 (I): Metabolome analysis under different trophic conditions

Yuki Kaniya<sup>1</sup>, Ayumi Kizawa<sup>1</sup>, Atsuko Miyagi<sup>2</sup>, Maki Kawai<sup>1,2</sup>, Hirofumi Uchimiya<sup>2</sup>, Yasuko Kaneko<sup>1,2</sup> and Yukako Hihara<sup>1,2,3</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Graduate School of Science and Engineering, Saitama University, Saitama, Japan; <sup>2</sup>Institute for Environmental Science and Technology, Saitama University, Saitama, Japan; <sup>3</sup>PRESTO, Japan Science and Technology Agency (JST), Saitama, Japan

Every cyanobacterial species possesses multiple genes encoding AbrB-like transcriptional regulators (CyAbrBs) distinct from those conserved among other bacterial species. Since multiple copies in a single cyanobacterium are classified into two different clades in most cases, we propose to call them *cyabrA* and *cyabrB*. We made and characterized a gene-disrupted mutant of *cyabrB* (slI0822) in *Synechocystis* sp. PCC 6803. The mutant showed many distinct phenotypes under ambient CO<sub>2</sub> conditions, indicating that CyAbrB is involved in the regulation of various cellular processes. First, the level of expression of nitrogen-regulated genes such as *urtA*, *amt1*, *glnB*, *sigE*, and the *nrt* operon was significantly lowered in  $\Delta$ *cyabrB*, although the induction of these genes upon nitrogen depletion was still observed to some extent [1]. Secondly, expression levels of genes encoding cytokinetic components such as FtsZ and FtsW were lowered in the  $\Delta$ *cyabrB* mutant [2]. The cell volume of the  $\Delta$ *cyabrB* cells was five times that of the wild-type cells and the proportion of dividing cells was notably higher in the mutant culture, indicating the defect in cell division. Thirdly, accumulation of glycogen granules was observed in the spaces between the thylakoid membranes in the mutant [2]. We observed that growth of the  $\Delta$ *cyabrB* mutant was severely inhibited after 12 h of incubation under photomixotrophic conditions with 5 mM glucose. When cells were observed by transmission electron microscopy, the  $\Delta$ *cyabrB* mutant looked blackish due to densely accumulated glycogen granules. In order to know what is happening to the central carbon metabolism in the mutant cells, we performed metabolome analysis using capillary electrophoresis mass spectrometry (CE/MS). When metabolite levels were compared before and after 12 h of incubation under photomixotrophic conditions, marked increase of the metabolites in glycolysis and oxidative pentose phosphate pathway were observed both in the wild-type and the  $\Delta$ *cyabrB* mutant cells. It was notable that amounts of pyruvate and 2-oxoglutarate (2-OG) in the mutant were significantly lower than those of the wild type irrespective of trophic condition. Carbon flow to TCA cycle seems to be restricted in the  $\Delta$ *cyabrB* mutant at the step of pyruvate synthesis. This may cause the aberrant accumulation of glycogen and shortage of 2-OG and nitrogen-containing metabolites synthesized from 2-OG.

[1] Ishii A, Hihara Y (2008) Plant Physiol. 148: 660-670; [2] Yamauchi Y, Kaniya Y, Kaneko Y, Hihara Y (2011) J. Bacteriol. 193: 3702-3709

## P94: Disruption of *slr2031* alleviates high light sensitive phenotype of a mutant lacking light-dependent protochlorophyllide reductase in the cyanobacterium *Synechocystis* sp. strain PCC 6803

**Yuto Hiraide and Yuichi Fujita**

Graduate School of Bioagricultural Sciences, Nagoya University

Biosynthesis of chlorophyll (Chl) requires at least 15 enzymatic reaction steps. For the penultimate step; the reduction of protochlorophyllide (Pchlde), most oxygenic photosynthetic organisms including cyanobacteria employ two structurally unrelated enzymes; light-dependent Pchlde oxidoreductase (LPOR) and dark-operative Pchlde oxidoreductase (DPOR). DPOR shows significant similarity to nitrogenase [1], which is rapidly inactivated by oxygen. This oxygen sensitive property is conserved in DPOR [2]. An LPOR-lacking mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 ( $\Delta por$ ) is not able to grow under high light conditions while  $\Delta por$  grows slowly under low light conditions. This high-light sensitive phenotype might be derived from the inactivation of DPOR by the higher production of oxygen, which is supported by the observation that  $\Delta por$  grows normally in low-oxygen conditions as well as the wild type even under high light. It is suggested that there are some unknown protection mechanism to allow DPOR operating under permissive light intensity. In this work we report that the disruption of *slr2031* alleviates the high light sensitive phenotype of  $\Delta por$ . *slr2031*, which encodes a putative phosphatase, is located at a neutral site of the genome. During preliminary study to use the neutral site for overexpression of candidate genes for the protection, we happened to find that the lack of *slr2031* correlates with the relief of the high light sensitive phenotype of  $\Delta por$ . Then we isolated a double mutant lacking both LPOR and Slr2031 ( $\Delta por/\Delta slr2031$ ).  $\Delta por/\Delta slr2031$  grew under higher light conditions than  $\Delta por$ . The  $\Delta por/\Delta slr2031$  cells contained significantly reduced amounts of Pchlde and other Chl intermediates accumulated in  $\Delta por$ , and the Chl content of  $\Delta por/\Delta slr2031$  was partially recovered, suggesting that DPOR activity is somewhat restored. In addition, we observed that the lack of *slr2031* caused decrease in phycocyanin content and increase in carotenoid contents, especially myxoxanthophyll. Semi-quantitative reverse transcriptase PCR analysis revealed that the increase of carotenoid content was achieved by enhanced mRNA levels of some *crt* genes in  $\Delta por/\Delta slr2031$  compared with  $\Delta por$ . Considering that the rate of carotenoid synthesis is enhanced in high light with preferential synthesis of myxoxanthophyll [3], it is implied that the disruption of *slr2031* causes artificially the high light response in  $\Delta por$ . Biosynthesis of chlorophyll (Chl) requires at least 15 enzymatic reaction steps. For the penultimate step; the reduction of protochlorophyllide (Pchlde), most oxygenic photosynthetic organisms including cyanobacteria employ two structurally unrelated enzymes; light-dependent Pchlde oxidoreductase (LPOR) and dark-operative Pchlde oxidoreductase (DPOR). DPOR shows significant similarity to nitrogenase [1], which is rapidly inactivated by oxygen. This oxygen sensitive property is conserved in DPOR [2]. An LPOR-lacking mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 ( $\Delta por$ ) is not able to grow under high light conditions while  $\Delta por$  grows slowly under low light conditions. This high-light sensitive phenotype might be derived from the inactivation of DPOR by the higher production of oxygen, which is supported by the observation that  $\Delta por$  grows normally in low-oxygen conditions as well as the wild type even under high light. It is suggested that there are some unknown protection mechanism to allow DPOR operating under permissive light intensity. In this work we report that the disruption of *slr2031* alleviates the high light sensitive phenotype of  $\Delta por$ . *slr2031*, which encodes a putative phosphatase, is located at a neutral site of the genome. During preliminary study to use the neutral site for overexpression of candidate genes for the protection, we happened to find that the lack of *slr2031* correlates with the relief of the high light sensitive phenotype of  $\Delta por$ . Then we isolated a double mutant lacking both LPOR and Slr2031 ( $\Delta por/\Delta slr2031$ ).  $\Delta por/\Delta slr2031$  grew under higher light conditions than  $\Delta por$ . The  $\Delta por/\Delta slr2031$  cells contained significantly reduced amounts of Pchlde and other Chl intermediates accumulated in  $\Delta por$ , and the Chl content of  $\Delta por/\Delta slr2031$  was partially recovered, suggesting that DPOR activity is somewhat restored. In addition, we observed that the lack of *slr2031* caused decrease in phycocyanin content and increase in carotenoid contents, especially myxoxanthophyll. Semi-quantitative reverse transcriptase PCR analysis revealed that the increase of carotenoid content was achieved by enhanced mRNA levels of some *crt* genes in  $\Delta por/\Delta slr2031$  compared with  $\Delta por$ . Considering that the rate of carotenoid synthesis is enhanced in high light with preferential synthesis of myxoxanthophyll [3], it is implied that the disruption of *slr2031* causes artificially the high light response in  $\Delta por$ . Taken together, it is suggested that Slr2031 is involved in the suppression of the *crt* genes and some other unknown genes for the protection of DPOR from oxygen under low light intensity, and that these genes are induced even under low light conditions due to the absence of Slr2031 resulting in alleviation of the high light sensitive phenotype of  $\Delta por$ .

[1] Muraki N *et al.* (2010) Nature 465: 110-114; [2] Yamamoto H *et al.* (2009) Plant Cell Physiol 50: 1663-1673; [3] Steiger S *et al.* (1999) J Photochem Photobiol 52: 14-18

## P95: Protochromic green/red photocycle of chromatic acclimation sensor proteins

**Yuu Hirose<sup>1</sup>, Nathan C. Rockwell<sup>2</sup>, Shelley S. Martin<sup>2</sup>, Kaori Nishiyama<sup>3</sup>, Rei Narikawa<sup>4</sup>, Yutaka Ukaji<sup>3</sup>, Katsuhiko Inomata<sup>3</sup>, Clark J. Lagarias<sup>2</sup> and Masahiko Ikeuchi<sup>4</sup>**

<sup>1</sup>Electronics-Inspired Interdisciplinary Research Institute (EIIRIS), Toyohashi University of Technology, Aichi, Japan; <sup>2</sup>Department of Molecular and Cellular Biology, University of California, Davis, CA; <sup>3</sup>Division of Material Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Ishikawa, Japan; <sup>4</sup>Department of Life Sciences (Biology), the University of Tokyo, Tokyo, Japan

Cyanobacteriochromes (CBCRs) are unique cyanobacterial members of the phytochrome superfamily of photosensory proteins. Like phytochrome, CBCRs reversibly photoconvert between two photostates, which is caused by photoisomerization of a linear tetrapyrrole (bilin) chromophore bound within a GAF domain. While phytochromes are normally red/far-red color sensors, CBCRs exhibit much more diverse photocycles, providing coverage of all visible and near ultraviolet colors. Perception of blue and ultraviolet colors has shown to be achieved by formation of "thiol-adduct" between the bilin and second conserved Cys residue, but molecular basis for longer wavelength such as green and red colors remains unknown. CcaS and RcaE are closely related CBCRs that regulate complementary chromatic acclimation (CCA) [1,2], in which cyanobacteria optimize composition of their photosynthetic antenna responding to green and red light. CcaS reversibly photoconverts between a green-absorbing state (Pg) and a red-absorbing state (Pr) [3], but RcaE has to date proven difficult to study *in vitro*. Here, we demonstrated that RcaE of *Fremyella diplosiphon* also undergo the same green/red photocycle. Moreover, we discovered that the green/red photocycle is protochromic: Pg formation is caused by deprotonation of bilin chromophore, whereas Pr formation is caused by protonation of the bilin. The proton transfer is triggered by bilin photoisomerization and requires three key residues that modulate pKa of the bilin. Thus, we elucidate the basis of the protochromic photocycle of CCA sensors, which provides new insight into our understanding how CBCRs tunes their spectral properties to sense various light colors.

[1] Kehoe, D. M. & Gutu, A. (2006) Responding to color: the regulation of complementary chromatic adaptation. *Annu. Rev. Plant. Biol.* 57, 127-150; [2] Hirose, Y., Narikawa, R., Katayama, M. & Ikeuchi, M. (2010) Cyanobacteriochrome CcaS regulates phycoerythrin accumulation in *Nostoc punctiforme*, a group II chromatic adapter. *Proc. Natl. Acad. Sci. U. S. A.* 107, 8854-8859; [3] Hirose, Y., Shimada, T., Narikawa, R., Katayama, M. & Ikeuchi, M. (2008) Cyanobacteriochrome CcaS is the green light receptor that induces the expression of phycobilisome linker protein. *Proc. Natl. Acad. Sci. U. S. A.* 105, 9528-9533

## P96: Impaired glycogen synthesis causes energy spilling reactions and affects stress responses in the *Cyanobacterium synechocystis* sp.

**Marianne Gründel, Ramon Scheunemann, Wolfgang Lockau and Yvonne Zilliges**

Humboldt-Universität zu Berlin, Institute of Biology/ Plant Biochemistry, Chausseestr. 117, 10115 Berlin

Many microorganisms accumulate polymers as carbon and energy reserves to cope with transient starvation conditions. Glycogen biosynthesis is a main strategy for such metabolic storage. The polymer usually accumulates in bacteria when an appropriate carbon source is available in sufficient amounts but growth is inhibited by lack of other nutrients. Cyanobacteria, algae and plants synthesize polyglucans during the day and use them as a respiratory substrate for dark maintenance. Therefore, a complex regulatory network might exist to adjust glycogen synthesis and degradation to cellular needs in phototrophic organisms.

This study of the phototrophic model organism *Synechocystis* sp. PCC 6803 elucidates the impact of glycogen metabolism on the response to major stresses that usually occur in natural environments, such as light-to-dark transitions and changes in macronutrient supply. Under conditions of energy and carbon excess, mutants impaired in glycogen synthesis by knockout of either ADP-glucose pyrophosphorylase or glycogen synthases show prominent phenotypes: light-induced glucose sensitivity (i), incapability of starvation-induced metabolic switch to the dormant state which typically includes degradation of the light-harvesting phycobilisome complex (ii) and energy-spilling reactions that are marked by a massive loss of 2-oxoglutarate and pyruvate (iii). We demonstrate that glycogen metabolism has an eminent role in viability maintenance and carbon and energy homeostasis. The results provide novel insights in regulatory interactions of the complex carbon metabolism and the metabolic control of stress-specific responses in cyanobacteria.

## P97: Mg-protoporphyrin oxidative cyclase in phototrophic *Proteobacteria*

**Zuzana Vávrová<sup>1,2</sup>, Ekaterina N. Boldareva-Nuyanzina<sup>1</sup>, Roman Sobotka<sup>1,2</sup> and Michal Koblížek<sup>1,2</sup>**

<sup>1</sup>Institute of Microbiology CAS, Dept. of Phototrophic Microorganisms - Algatech, Opatovický mlýn, 379 81 Třeboň, Czech Republic; <sup>2</sup>Faculty of Science, University of South Bohemia, 370 00 České Budějovice, Czech Republic

Magnesium-protoporphyrin IX monomethylester cyclase is one of key enzymes in (bacterio)chlorophyll biosynthesis. It catalyses formation of the fifth ring of tetrapyrrole molecule. Despite of the importance of the fifth ring for the bacterio(chlorophyll) function, its formation is not completely understood. There exist two fundamentally different forms of the enzyme: an oxygen-dependent cyclase operating under aerobic conditions and an oxygen-independent cyclase extracting the oxygen atom from water, for which subunits code genes *acsF* and *bchE*, respectively. First, we performed a detailed survey of evolutionary old phototrophic *Proteobacteria* for the presence of *acsF* or *bchE* genes. Using available genomic data and newly designed degenerated primers this survey revealed that the majority (50 out of 59 species tested) contained *acsF* gene. The high sequence similarity of *acsF* gene with its cyanobacterial and algal counterparts suggests that *Proteobacteria* might have recruited this gene via horizontal gene transfer from *Cyanobacteria* during the Proterozoic period. This is also indicated by the recognition of proteobacterial AcsF proteins by polyclonal antibody raised against its plastidial homologue from *Arabidopsis thaliana*. Further we focus on the mechanism of the fifth ring cyclization. The conversion of magnesium-protoporphyrin IX monomethylester into divinyl protochlorophyllide consists of three sequential steps. We assume that AcsF protein requires for its proper function additional proteins. We therefore aim to find and characterize these putative binding partner(s) of AcsF protein from our model organism *Erythrobacter* NAP1 and undergo reverse genetic tools on the background of *Synechocystis* PCC 6803 mutants.

## P98: A novel protein exclusive of filamentous cyanobacteria involved in cellular division

**Dinka Mandaković<sup>1</sup>, Carla Trigo<sup>1</sup>, José Enrique Frías<sup>2</sup>, Enrique Flores<sup>2</sup> and Mónica Vásquez<sup>1</sup>**

<sup>1</sup>Laboratorio de Ecología Microbiana y Toxicología Ambiental, Departamento de Genética Molecular y Microbiología, Pontificia Universidad Católica de Chile, 6513492, Santiago, Chile; <sup>2</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, Consejo Superior de Investigaciones Científicas y Universidad de Sevilla, Américo Vespucio 49, E-41092, Sevilla, España

Some filamentous cyanobacteria are multicellular organisms that contain differentiated cells with specialized functions, including vegetative cells that perform oxygenic photosynthesis and heterocysts that fix nitrogen [1]. In the genomic comparative study done by Stucken and coworkers [2], a group of 10 genes was identified as exclusive of filamentous cyanobacteria. One gene of this group is *all2320* from *Anabaena* sp. PCC 7120, which encodes a protein of unknown function that bears one transmembrane segment and one coiled coil domain, and therefore could have a membrane-associated function in filamentous cyanobacteria. Studying its genetic context in different filamentous cyanobacteria and experimentally by northern blot analysis in *Anabaena* sp. PCC 7120, we observed that this gene is monocistronic. When analyzing gene expression in *Anabaena* sp. PCC 7120 by RT-qPCR, we observed a constitutive expression of *all2320*, either in the absence and presence of nitrogen in the culture media until 72 hours of growth, very similar to the expression observed for *ftsZ* (cell division gene). To localize the protein in the cell, All2320 was expressed in *Escherichia coli* and purified, and an anti-All2320 antibody was generated. Using immunofluorescence labeling and confocal microscopy, we could observe that All2320 seems to localize at one cell pole during cell division, being first observed in the membrane of growing cells, probably before the Z-ring is formed, and then staying in only one of the sister cells mainly in one pole of that cell. To get more information of this protein and its function, mutant strains bearing inactivated copies of *all2320* have been generated. Although not fully segregated, an altered cell division, with production of cells showing division in more than one plane, has been observed in these strains. These results suggest a function of All2320 during cell division and related to filament growth.

[1] Flores, E.; Herrero, A. 2010. Compartmentalized function through cell differentiation in filamentous cyanobacteria. *Nat Rev Microbiol.* 8:39-50; [2] Stucken, K.; John, U.; Cembella, A.; Murillo, A.; Soto-Liebe, K.; Fuentes, J.; Friedel, M.; Plominsky, AM.; Vásquez, M.; Glockner, G. 2010. The smallest known genomes of multicellular and toxic cyanobacteria: comparison, minimal gene sets for linked traits and the evolutionary implications. *PLoS One* 5:e9235.

## P99: Altered regulatory RNA metabolism: Biochemical and physiological analysis

**Carla Albert R.R. Rosana<sup>1</sup>, Jens Georg<sup>2</sup>, Danuta Chamot<sup>1</sup>, Wolfgang R. Hess<sup>2</sup>, George W. Owtrim<sup>1</sup>**

<sup>1</sup>Lab 1Department of Biological Sciences, University of Alberta, Edmonton Alberta Canada T6G 2E9; <sup>2</sup>Institut f. Genetik, Universität Freiburg, Germany

DEAD-box RNA-helicases are ubiquitous enzymes whose rearrangement of RNA secondary structure modulates gene expression and RNP activity. Specific RNA targets for RNA helicases have rarely been described. A single DEAD-box RNA helicase, CrhR (slr0083), is encoded in the cyanobacterium *Synechocystis* sp. PCC 6803 genome, whose inactivation has drastic physiological effects particularly under cold stress. In order to identify the RNA targets of CrhR, custom microarrays containing sequences corresponding to non-coding small RNAs (5'UTRs, ncRNAs, asRNAs) and all mRNAs identified a specific segment of the transcriptome whose expression is altered in the absence of functional CrhR RNA helicase activity. CrhR-dependent, differential expression of <200 RNA elements was detected, including both mRNA and small RNA transcripts. While mRNA expression changed randomly, >90% of the affected small RNAs increased in abundance in a temperature specific manner, at 20°C, in response to *crhR* mutation. We have validated the microarray data and determined target RNA transcript half-life that indicate CrhR does not function in transcript turnover. Biochemical assays reveal that CrhR can anneal but not unwind identified mRNA-asRNA duplexes. The data indicate that CrhR RNA helicase activity performs a distinct role in small RNA metabolism, associated with the regulation of expression of a specific subset of the total *Synechocystis* transcriptome. This CrhR-controlled RNA regulon may perform roles in cyanobacterial acclimation to low temperature. Investigations are in progress to identify the role CrhR performs in small RNA metabolism and temperature acclimation.



## ***P100: Quorum-sensing in the purple alpha-proteobacterium *Rhodobacter capsulatus****

**Cedric A. Brimacombe and J. Thomas Beatty**

Department of Microbiology & Immunology, University of British Columbia, Vancouver, BC, Canada

A large proportion of the proteobacteria utilize LuxI/R type quorum-sensing (QS) systems, where a LuxI-type of protein synthesizes an acyl-homoserine lactone signalling molecule (acyl-HSL) and a LuxR-type of protein binds the acyl-HSL to either activate or repress gene transcription. Therefore cells communicate by the production of and response to acyl-HSLs, and this communication changes activities within microbial communities. The GtaI and GtaR proteins in *Rhodobacter capsulatus* are homologous to LuxI/R-type proteins, and form a QS system that regulates genetic exchange and the production of extracellular polysaccharide. The *gtaR/I* genes are co-transcribed, are autoregulatory, and form an autoinducing circuit. The GtaR protein is a negative regulator of transcription, and modulates expression of the gene transfer agent (RcGTA), and transcription of the response regulator *ctrA* gene and the *gtaRI* operon. Purified GtaR readily binds the *gtaRI* promoter but does not bind to the RcGTA or *ctrA* promoter, and so regulation of RcGTA and *ctrA* appears to be indirect, therefore implicating at least one other transcriptional regulator. Observable phenotypes and microarray analysis of relative gene expression in GtaR/I QS mutants versus wild type cells indicate that the GtaR/I QS system regulates a number of genes in the *R. capsulatus* genome. Genetic and biochemical approaches revealed that the GtaR protein interacts with a variety of acyl-HSLs, and therefore may communicate with a variety of species in microbial communities [1]. Our latest research indicates that the GtaR/I QS system also regulates the production of extracellular polysaccharide.

[1] Leung, M.M., C.A. Brimacombe, G. B. Spiegelman and J.T. Beatty (2012). The GtaR protein negatively regulates transcription of the *gtaRI* operon and modulates gene transfer agent (RcGTA) expression in *Rhodobacter capsulatus*. *Molec. Microbiol.* 83:759-774

## ***P101: Functional analysis of additional circadian clock proteins in the fresh water cyanobacterium *Synechocystis* sp. PCC 6803***

**Julia Holtzendorff, Anja K. Bäcker, Hans-Tobias Deinzer, Annegret Wilde**

Institute for Microbiology and Molecular Biology, Justus Liebig University, Giessen, Germany

The core oscillator of the circadian clock in cyanobacteria consists of 3 proteins, KaiA, KaiB, and KaiC. All 3 are essential for clock function. As in the case of the model organism of cyanobacterial circadian research, *Synechococcus elongatus* PCC 7942, most cyanobacteria possess only one copy of each kai gene.

One exception is the marine genus *Prochlorococcus*, which has lost its kaiA gene, resulting in an hourglass-like clock instead of a robust circadian clock [1]. Another exception is the fresh water strain *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) that harbors multiple *kai* genes: In addition to the classical *kaiABC* gene cluster, this strain possesses a *kaiC2B2* operon and orphan *kaiC3* and *kaiB3* genes. The robustness of the circadian rhythms of *Synechocystis* is significantly lower than that of *Synechococcus* [2].

We are currently characterizing all *Synechocystis* clock proteins in order to understand their function and potential implication on clock robustness. Our *in vivo* interaction studies show that among the Kai proteins, interaction occurs only between the classical KaiB and KaiC proteins that resemble most those of the model strain *Synechococcus elongatus* PCC 7942. In contrast, KaiC2, a KaiC-homologue that is also present in purple bacteria seems to interact with components of the phycobilisome and the RNA polymerase. Deletion studies indicate, that either KaiB2 or KaiC2 or both are essential proteins, since knockout mutants are not viable.

In the classical *Synechococcus* sp. PCC 7942 system, autophosphorylation of KaiC is enhanced by KaiA and reduced by KaiB. The same was found for the *Synechocystis* KaiABC proteins but not for KaiC3 (see Poster A. Wiegand).

Our data suggest that the additional Kai proteins of *Synechocystis* might not be involved in circadian regulation. KaiB2 and KaiC2 seem to be essential proteins and understanding their role in *Synechocystis* could also shed light on the function of potential KaiB2/KaiC2 systems in purple bacteria.

[1] Holtzendorff J, Partensky F, Mella D, Lennon JF, Hess WR, Garczarek L. Genome streamlining results in loss of robustness of the circadian clock in the marine cyanobacterium *Prochlorococcus marinus* PCC 9511. *J Biol Rhythms.* 2008 Jun;23(3):187-99; [2] Aoki S, Kondo T, Ishiura M. Circadian expression of the *dnaK* gene in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *J Bacteriol.* 1995 Oct;177(19):5606-11



## P102: Experimental and Modeling Analysis of *Synechocystis* sp. PCC 6803 Growth

**Miguel Lopo<sup>1</sup>, Arnau Montagud<sup>5</sup>, Emilio Navarro<sup>4</sup>, Isabel Cunha<sup>1</sup>, Andrea Zille<sup>1</sup>, Pedro Fernández de Córdoba<sup>5</sup>, Pedro Moradas-Ferreira<sup>1,2</sup>, Paula Tamagnini<sup>1,3</sup> and Javier F. Urchueguía<sup>5</sup>**

<sup>1</sup>IBMC – Instituto de Biologia Molecular e Celular, <sup>2</sup>Instituto de Ciências Biomédicas Abel Salazar (ICBAS); <sup>3</sup>Departamento de Botânica, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; <sup>4</sup>Departamento de Lenguajes y Ciencias de la Computación, Campus de Teatros, Universidad de Málaga, Málaga; <sup>5</sup>Instituto de Matemática Pura y Aplicada, Universidad Politécnica de Valencia, Valencia, Spain

Many physiological studies have addressed the effects of environmental factors affecting cyanobacteria growth and *Synechocystis* sp. PCC 6803 in particular. However, multifactorial studies are scarce. In this work, the influence of different parameters such as temperature, irradiance, nitrate concentration, pH, and an external carbon source on *Synechocystis* growth was evaluated [1]. A high-throughput system equivalent to batch cultures, with 4.5 ml cuvettes containing 2 ml of culture, was used. Gas exchange was ensured by the use of a Parafilm™ cover. The effect of the different variables on maximum growth was assessed by a multi-way statistical analysis. Temperature and pH were identified as the key factors. It was observed that *Synechocystis* cells have a strong influence on the external pH. The optimal growth temperature, for these experiment settings, was 33°C while light-saturating conditions were reached at 40  $\mu\text{E m}^{-2} \text{s}^{-1}$ . It was demonstrated that *Synechocystis* exhibits a marked difference in behavior between autotrophic and glucose-based mixotrophic conditions, and that for the duration of the experiments, nitrate concentrations did not have a significant influence on growth, probably due to endogenous nitrogen reserves. Furthermore, a dynamic metabolic model of *Synechocystis* photosynthesis was developed to gain insights on the underlying mechanism enabling this cyanobacterium to control the levels of external pH. The model showed a coupled effect between the increase of the pH and ATP production which in turn allows a higher carbon fixation rate.

[1] Lopo *et al.* (2012) *J. Mol. Microbiol. Biotechnol.* 22:71-82.

## P103: Role of HupW and HoxW on the maturation of hydrogenases in *Anabaena* sp. PCC7120

**Paulo Oliveira<sup>1</sup>, Miguel Lopo<sup>1</sup>, Filipe Pinto<sup>1,2</sup>, Sérgio Ferreira<sup>1</sup>, Catarina C. Pacheco<sup>1</sup>, and Paula Tamagnini<sup>1,2</sup>**

<sup>1</sup>IBMC - Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal; <sup>2</sup>Faculty of Sciences, Department of Biology, University of Porto, Porto, Portugal

The genome of *Anabaena* sp. strain PCC 7120 contains genetic information coding for structural subunits of two types of Ni-Fe hydrogenases: an uptake (HupSL) and a bidirectional (HoxEFUYH) hydrogenase. Extensive physiological data has been collected over the years demonstrating the presence and activity of both enzymes. According to the model of Ni-Fe hydrogenase maturation and assembly based on what is known in *Escherichia coli*, accessory proteins (Hyp) are necessary to convert the hydrogenase apoprotein into an active enzyme. During this stage, the Ni-Fe active center and respective ligands are properly inserted into the hydrogenase large subunit. The final maturation step consists of a proteolytic cleavage at the C-terminus of the hydrogenase large subunit, elicited by an endopeptidase that has the ability to bind to nickel. This step can be considered a maturation checkpoint, where only hydrogenases possessing nickel and iron atoms in their active centers will be recognized by the endopeptidase and further cleaved, securing the proper assembly and functionality of the hydrogenase; thus, hydrogenase apoproteins lacking nickel will not be cleaved. Based on sequence homology, two putative endopeptidases have been suggested to be involved in the maturation of HupSL and HoxEFUYH in *Anabaena* sp. PCC 7120, namely HupW and HoxW. Recent data suggests that HupW specifically cleaves HupL [1]. However, some questions remain unanswered: is HoxH specifically cleaved by HoxW, or can its maturation be completed by another peptidase? Does the transcription of the hydrogenase structural genes follow the same expression pattern as the putative endopeptidases, and are their relative expression levels comparable? Is the transcription localized or is it equally spread throughout the filament? Can HupW and HoxW have additional targets? In order to clarify these questions and gain a better insight into the hydrogen metabolism of *Anabaena* sp. PCC 7120 we have started by constructing mutants lacking *hoxW* and *hupW/hoxW*. Their phenotypes are being evaluated in relation to the wild-type as well as to a strain lacking *hypF*, which is involved in earlier stages of hydrogenase maturation. Localization of gene expression is being studied using promoter-GFP fusions, addressing the specific expression not only of hydrogenase putative proteases genes *hupW* and *hoxW*, but also the hydrogenase structural genes *hupSL*, *hoxEF* and *hoxUYH*. Moreover, quantitative gene expression is currently being performed by RT-qPCR using RNA extracted from cells grown under different conditions. Western blot analyses, making use of different sets of newly developed antibodies raised against both hydrogenases, will aid evaluating the specificity of each endopeptidase. Our most recent results will be presented and further discussed.

[1] Lindberg, P. Devine, E., Stensjö, K. and Lindblad, P. (2012) *Appl Environ Microbiol*, 78, 273-276.

## P104: Involvement of Cyclic GMP in controlling *Rhodospirillum centenum* cyst development

Qian Dong<sup>1</sup>, Sugata Roy Chowdhury<sup>1</sup>, Jeremiah Marden<sup>2</sup> and Carl Bauer<sup>1</sup>

<sup>1</sup>Molecular and Cellular Biochemistry Department, Indiana University- Bloomington, IN, 47405; <sup>2</sup>Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC, 27599

*Rhodospirillum centenum* is a purple photosynthetic  $\alpha$ -proteobacterium with a complex developmental life-cycle featuring three morphologically distinct cell types; swim cells, swarm cells and resting cysts. Cysts are metabolically dormant as a means of surviving environmental stresses such as desiccation. Wild-type *R. centenum* excretes large amounts of cGMP when transitioning from vegetative growth into encystment, whereas a strain deleted for recently characterized guanylyl cyclase fails to synthesize cGMP and is severely disrupted in cyst-cell maturation. The cyst defective phenotype of the cyclase mutation can be fully complemented by exogenously added cGMP. There are also two open reading frames, *rc1\_3786* and *rc1\_3787* linked to the *R. centenum* guanylyl cyclase. To explore the function of these additional genes we constructed in frame deletions of ORFs *rc1\_3786* and *rc1\_3787* in *R. centenum*. Deletions of either *rc1\_3786* or *rc1\_3787* results in a phenotype that is virtually indistinguishable from the strain deleted for the cyclase. Specifically, these additional deletion strains are defective in synthesis of cGMP and in forming cysts. The defect in cyst development can be complemented by the exogenous addition of cGMP.

A homologue of the *E. coli* cAMP receptor protein (CRP) is linked to the guanylyl cyclase and when deleted results in a deficiency in cyst development and cGMP secretion. Isothermal calorimetry (ITC) and differential scanning fluorimetry (DSF) analyses demonstrate that the recombinant CRP homologue preferentially binds to, and is stabilized by cGMP. qPCR experiments show that expression levels of both *rc1\_3786* and *rc1\_3787* are largely elevated when cell is cultured with exogenous cGMP. However, strain deleted of CRP homolog does not show similar increased expression. Our working model for cGMP signaling in *R. centenum* involves the activation of the CRP homolog *rc1\_3788* in response to the presence of cGMP that subsequently induces expression of guanylyl cyclase catalytic subunit as well as *rc1\_3786* and *rc1\_3787*.

The results of our study provide evidence that cGMP has a crucial role in regulating prokaryotic development. The involvement of cGMP in regulating bacterial development has broader implications as several plant-interacting bacteria contain a similar cyclase coupled by the observation that *Azospirillum brasilense* also synthesizes cGMP when inducing cysts. In order to further explore how the CRP homolog can modulate cyst formation we are currently performing whole genome DNA tiling array on *R. centenum* of different developmental stage: from vegetative cells to cyst cells and will report any relevant array data obtained prior to the conference.

## P105: Multiple circadian clock proteins in *Synechocystis* sp. PCC 6803

Anika Wiegand<sup>1</sup>, Anja K. Bäcker<sup>2</sup>, Annegret Wilde<sup>2</sup>, and Ilka M. Axmann<sup>1</sup>

<sup>1</sup>Charité Universitätsmedizin und Humboldt-Universität zu Berlin, Institut für Theoretische Biologie, Invalidenstraße 42, 10115 Berlin, Germany;

<sup>2</sup>Justus-Liebig-Universität Giessen, Institut für Mikrobiologie und Molekularbiologie, Heinrich-Buff-Ring 26, 35392 Giessen, Germany

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 possess 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 *kaiA* 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 kinase activity of KaiC3 seems to be 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.

[1] Brettschneider C, Rose RJ, Hertel S, Axmann IM, Heck AJ, Kollmann M. (2010) A sequestration feedback determines dynamics and temperature entrainment of the KaiABC circadian clock. *Mol Syst Biol.* 6:389; [2] Axmann IM, Dühring U, Seeliger L, Arnold A, Vanselow JT, Kramer A, and Wilde A (2009) Biochemical evidence for a timing mechanism in *prochlorococcus*. *J Bacteriol.* 191(17):5342-7

**P106: Photoperiod length shapes the carbon-nitrogen metabolism in *Crocospaera watsonii*****Anthony Dron<sup>1,2</sup>, Sophie Rabouille<sup>1,2</sup>, Pascal Claquin<sup>3,4</sup>, and Antoine Sciandra<sup>1,2</sup>**<sup>1</sup>UPMC Univ Paris 06, UMR 7093, LOV, Observatoire Océanologique de Villefranche-sur-Mer, F-06234, Villefranche-sur-Mer, France; <sup>2</sup>CNRS, UMR 7093, LOV, Observatoire Océanologique, F-06234, Villefranche-sur-Mer, France; <sup>3</sup>Université Caen Basse-Normandie, FRE BIOMEA, F-14032 Caen, France; <sup>4</sup>CNRS INEE, FRE BIOMEA, F-14032 Caen, France

Carbon-nitrogen (C, N) metabolism and growth in photoautotrophic organisms are constrained by environmental factors, such as the light availability or photoperiod length (PPL). Owing to the O<sub>2</sub>-sensitivity of the N<sub>2</sub>-fixing nitrogenase enzyme, C and N acquisition in the unicellular, N<sub>2</sub>-fixing cyanobacterium *Crocospaera watsonii* are separated in time: N<sub>2</sub> fixation is restricted to the dark period when oxygenic photosynthesis does not occur [1]. Using continuous-culture devices [2], we propose to tackle the effect of short-, intermediate- and long-PPL (8:16, 12:12 and 16:8 light-dark conditions, respectively) on the growth and the C-N metabolism of the worldwide, important N<sub>2</sub>-fixer [3,4], *C. watsonii* WH8501 [5]. When cultures were at the equilibrium, samples were collected at high frequency during four consecutive days. These experiments revealed a daily cycle in the physiological and biochemical parameters, tightly constrained by the timely decoupled processes of N<sub>2</sub> fixation and C acquisition. N<sub>2</sub>-fixation occurs mostly, but not exclusively during the dark period and leads to an increase in the N cell content while the C cell content, accumulated during light period, decreases to supply energy. We observed a trade-off in the energy allocation: in short-PPL, the somatic growth is fostered whereas in long-PPL the cellular growth is accelerated. One of our most striking results is that N<sub>2</sub>-fixation exceeded 1.3 to 3-fold and 2.3 to 4.9-fold the N requirements for growth in intermediate- and short-PPL respectively. These observations suggest that a large amount of N (eg. ammonium or dissolved organic N) is excreted into the environment: a point discussed in regard to nitrogen cycling in large N-depleted, oceanic gyres.

[1] Mohr, W., Intermaggio, M.P., and LaRoche, J. (2009) Diel rhythm of nitrogen and carbon metabolism in the unicellular, diazotrophic cyanobacterium *Crocospaera watsonii* WH8501. *Environ Microbiol* 12: 412-421; [2] Malara, G., and Sciandra, A. (1991) A multiparameter phytoplanktonic culture system driven by microcomputer. *J Applied Phycol* 3: 235-241; [3] Zehr, J.P., Waterbury, J.B., Turner, P.J., Montoya, J.P., Omoregie, E., Steward, G.F., et al. (2001) Unicellular cyanobacteria fix N<sub>2</sub> in the subtropical North Pacific Ocean. *Nature* 412: 635-638; [4] Montoya, J.P., Holl, C.M., Zehr, J.P., Hansen, A., Villareal, T., and Capone, D.G. (2004) High rates of N<sub>2</sub>-fixation by unicellular diazotrophs in the oligotrophic Pacific. *Nature* 430: 1027-1031; [5] Waterbury, J.B., and Rippka, R. (1989) Cyanobacteria. Subsection I. Order Chroococcales. In: Krieg NR, Holt JB (eds). *Bergey's Manual of Systematic Bacteriology*. Vol.3. Williams and Wilkins: Baltimore. pp 1728-1746

**P107: Assaying interactions of the PixD and PixE components of the *Synechocystis* sp. PCC6803 phototaxis signaling pathway with Slr1692****Arjan Vermeulen & Carl Bauer**

Dept. of Molecular and Cellular Biochemistry, Indiana University, Bloomington IN

PixD is a short BLUF protein that has been shown to play a role in regulating phototaxis in *Synechocystis* sp. PCC6803. PixD has also been shown to interact with the PatA homologue PixE, suggesting the involvement of a multi-component signaling mechanism that responds to light intensity [1-3]. However, there is no mechanism established for how the PixD-PixE system promotes a downstream signal that controls phototaxis.

Recently, we studied the potential involvement of a third protein, Slr1692, encoded by an ORF upstream of *pixD* and *pixE*. This protein features an EAL domain and is therefore most likely involved in regulating the synthesis of the signaling molecule c-di-GMP. Using a bacterial-two-hybrid screen, we found evidence that PixD and PixE are indeed able to interact with Slr1692. We also found that Slr1692-PixE and PixD constitute an operon where all three ORFs are cotranscribed. Finally, we are in the process of disrupting all three of these ORF's with preliminary data suggesting that Slr1692 does indeed play a role in phototaxis as does PixD and PixE.

To date several additional receptors have been found to play a role in regulating phototaxis in *Synechocystis*. Some of these (PixJ1 and UirS) involve two-component systems, and one (Cph2) seems to work through a c-di-GMP system [4-6]. The PixD-PixE system may constitute a third independent pathway that also functions by controlling c-di-GMP or potentially by connecting to the Cph2 pathway.

[1] Yuan H, Bauer C. PixE promotes dark oligomerization of the BLUF photoreceptor PixD. *Proceedings of the ...* 2008 Aug. 1;105(33):11715-9; [2] Okajima K, Yoshihara S, Fukushima Y, Geng X, Katayama M, Higashi S, et al. Biochemical and functional characterization of BLUF-type flavin-binding proteins of two species of cyanobacteria. *Journal of Biochemistry*. 2005;137(6):741-50; [3] Masuda S, Hasegawa K, Ohta H, Ono T. Crucial Role in Light Signal Transduction for the Conserved Met93 of the BLUF Protein PixD/Slr1694. *Plant Cell Physiol*. 2008 Oct. 1;49(10):1600-6; [4] Bhaya D, Takahashi A, Grossman A. Light regulation of type IV pilus-dependent motility by chemosensor-like elements in *Synechocystis* PCC6803. *Proceedings of the ...* 2001;98(13):7540-5; [5] Song J-Y, Cho HS, Cho J-I, Jeon J-S, Lagarias JC, Park Y-I. Near-UV cyanobacteriochrome signaling system elicits negative phototaxis in the cyanobacterium *Synechocystis* sp PCC 6803. *Proceedings of the National Academy of Sciences*. 2011;108(26):10780-5; [6] Wilde A, Fiedler B, Borner T. The cyanobacterial phytochrome Cph2 inhibits phototaxis towards blue light. *Molecular Microbiology*. 2002;44(4):981-8

## P108: Sucrose degradation in salt-treated cyanobacteria

**Carolina N. Nishi<sup>1</sup>, María A. Kolman<sup>1</sup>, Walter A. Vargas<sup>1,2</sup> and Graciela L. Salerno<sup>1</sup>**

<sup>1</sup>CEBB-INBA-CONICET, CIB, FIBA, Mar del Plata, Argentina; <sup>2</sup>Centro Hispano Luso de Investigaciones Agrarias, CIALE, Universidad de Salamanca, Spain; Corresponding authors: gsalerno@fiba.org.ar, cnishi@fiba.org.ar

All organisms have to cope to the constantly changing environment in order to survive, and cyanobacteria are by no means the exception to that. In particular, cyanobacteria can grow under a wide range of environmental conditions, including salinity. Stenohaline cyanobacteria (with low salt tolerance) accumulate various low molecular osmolytes mainly sucrose (Suc), trehalose and sucroglucans. Suc is one of the most abundant disaccharide in nature and was reported to be present in oxygenic photosynthetic organisms. In particular, Suc metabolism has been biochemically, functionally and physiologically characterized in unicellular and heterocyst-forming cyanobacterial strains. In *Anabaena* sp. PCC 7120, Suc can be hydrolysed by two alkaline-neutral invertase isoforms (Inv-A and Inv-B) [1,2] or cleaved by sucrose synthase (SuS) [3]. In contrast, in the unicellular strain *Microcystis aeruginosa* PCC 7806 only SuS is responsible of Suc catabolism [4]. The aim of this study was to investigate the effect of a salt treatment on the expression of Suc degradative proteins and the role of Inv-A and Inv-B in *Anabaena* cells submitted to a salt stress. The expression of SuS or Inv encoding genes increased after salt addition. Also, Inv-A or Inv-B were not essential to cope the stress, when the growth of insertional mutants (lacking Inv-A or Inv-B) was determined. However, the accumulation of sucroglucans and glycogen was affected by the presence of NaCl. In the case of *M. aeruginosa*, both Suc accumulation and Suc cleavage by SuS increased with the salt treatment, indicating a sugar cycling during the stress. Taken together, our results suggest that Suc is not a mere salt-response osmolyte, and that a Suc cycling mechanism may be operating in both filamentous heterocyst-forming and unicellular bloom-forming strains in response to salinity.

[1] Vargas *et al.*, 2003. *Planta* 216, 951-960 (2003); [2] Vargas *et al.*, *Planta* 233, 153-162 (2011); [3] Curatti *et al.*, *FEBS Lett.* 513, 175-178 (2002); [4] Kolman *et al.*, *Planta* 235, 955-964 (2012)

## P109: The Novel Shell Protein CsoS1D is Essential to Carboxysome Function and Plays a Role in RubP Flux Across the Shell

**Evan W. Roberts<sup>1</sup>, Fei Cai<sup>2</sup>, William Hirst<sup>3</sup>, Anna A. Veldkamp<sup>4</sup>, Cedric D. Crump<sup>5</sup>, Cheryl A. Kerfeld<sup>2,6</sup>, Gordon C. Cannon<sup>1</sup> and Sabine Heinhorst<sup>1</sup>**

<sup>1</sup>Department of Chemistry and Biochemistry, University of Southern Mississippi, Hattiesburg, Mississippi, USA; <sup>2</sup>DOE Joint Genome Institute, Walnut Creek, California, USA; <sup>3</sup>Biochemistry Program, Vassar College, Poughkeepsie, New York, USA; <sup>4</sup>Department of Chemistry, Georgia Southern University, Statesboro, Georgia, USA; <sup>5</sup>Department of Natural Sciences and Environmental Health, Mississippi Valley State University, Itta Bena, Mississippi, USA; <sup>6</sup>Department of Plant and Microbial Biology, University of California, Berkeley, California, USA

Cyanobacteria and many chemoautotrophs efficiently convert carbon dioxide into metabolically useful organic compounds through the encapsulation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) in carboxysomes. The catalytic advantage RubisCO derives from being confined to the carboxysome is attributed to the shell-associated carbonic anhydrase, which rapidly equilibrates cytosolic bicarbonate to the RubisCO substrate CO<sub>2</sub>, and to the protein shell, which is thought to present a diffusional barrier that traps CO<sub>2</sub> in the carboxysome interior. Previously, all carboxysome components in chemoautotrophs and  $\alpha$ -cyanobacteria were believed to be accounted for by the genes of the *cso* operon, which encode the two Form 1A RubisCO subunits, the shell-associated carbonic anhydrase, and all known structural proteins of the shell. However, purification and characterization of the *Prochlorococcus marinus* MED4 (PMM) carboxysome established the presence of the tandem-BMC domain protein CsoS1D, which is encoded by a gene outside of the canonical *cso* operon, in the carboxysome shell [1]. Since *csoS1D* orthologs are present in the genomes of all carboxysome-forming chemoautotrophs and PMM is largely recalcitrant to genetic manipulation, the presence of CsoS1D in the carboxysomes of the model chemoautotroph *Halothiobacillus neapolitanus* was ascertained. To test the possibility that the central pore of the double-layered CsoS1D pseudo-hexamer mediates and/or regulates the transfer of ribulose 1,5-bisphosphate (RubP) across the carboxysome shell [2], an *H. neapolitanus* *csoS1D* deletion mutant was generated. The mutant exhibits the high CO<sub>2</sub>-requiring phenotype that is indicative of impaired carboxysome function. Although morphologically indistinguishable from their wild type counterparts, mutant carboxysomes that are devoid of CsoS1D have a similar apparent K<sub>c</sub> as wild type organelles but exhibit an approximately 50% higher apparent K<sub>m</sub> for RubP. These results suggest that CsoS1D plays a role in RubP transfer. Whether the pores in the CsoS1D pseudo-hexamers provide a gated entry of RubP into the carboxysome interior [2] remains to be determined. Clearly, the protein complement of carboxysomes is more complex than originally assumed based on the gene content of the classical *cso* operon.

[1] Evan W. Roberts, Fei Cai, Cheryl A. Kerfeld, Gordon C. Cannon and Sabine Heinhorst. Isolation and Characterization of the *Prochlorococcus* Carboxysome Reveal the Presence of the Novel Shell Protein CsoS1D. *Journal of Bacteriology*. 194, 787-795 (2012); [2] Michael G. Klein *et al.* Identification and Structural Analysis of a Novel Carboxysome Shell Protein with Implications for Metabolite Transport. *Journal of Molecular Biology*. 392, 319-333 (2009)

## **P110: Heterologous Expression of gene product all1371 in *E. coli* and Properties of a *Nostoc* PCC 7120 polyphosphate glucokinase mutant**

**Friederike Klemke, Thomas Volkmer, Linda Sawade, Ali Saitov and Wolfgang Lockau**

Plant Biochemistry, Humboldt-Universität zu Berlin, Chausseestrasse 117, Berlin, Germany

Cyanobacteria are a widespread group of oxygenic photoautotrophs synthesizing various secondary metabolites to cope with different stress conditions like nutrient deprivation. Polyphosphate a linear polymer of orthophosphate linked by energy-rich phosphoanhydride bonds appears to serve as a storage compound for inorganic phosphate and as an energy reservoir similar to ATP. In cyanobacteria polyphosphate is stored as granules in the cytoplasm of cells. We are interested in the function and in metabolism of this biopolymer in cyanobacteria. Our research focuses on the role of polyphosphate of unicellular and filamentous cyanobacteria. We are interested in the biochemical characteristics and the physiological relevance of enzymes involved in polyphosphate metabolism like polyphosphate kinase and polyphosphate glucokinase especially of diazotrophic cyanobacteria. The genome of *Nostoc* PCC 7120 a filamentous heterocyst-forming, nitrogen fixing cyanobacteria contains a gene that encodes a polyphosphate glucokinase (orf *all1371*) additionally to hexokinase. Both enzymes catalyze the phosphorylation of glucose to glucose-6-phosphate whereas the first uses polyphosphate as a phosphoryl-group donor and hexokinase utilizes ATP.

To get insight into the physiological role of polyphosphate glucokinase in diazotrophic cyanobacteria, a mutant of *Nostoc* PCC7120 was generated in which orf *all1371* was interrupted by mutagenesis. Polyphosphate was stained by DAPI [1] and visualized by fluorescence microscopy. Micrographs taken from mutant revealed an altered amount of polyphosphate compared to the wild type. Growth of *Nostoc* wild type and mutant was analyzed under various nitrogen and light conditions. Additionally polyphosphate glucokinase of *Nostoc* 7120 was heterologously expressed in *E. coli*, purified and characterized *in vitro*.

[1] Kulakova, A. N., D. Hobbs, *et al.* (2011). "Direct quantification of inorganic polyphosphate in microbial cells using 4'-6-diamidino-2-phenylindole (DAPI)." *Environ Sci Technol* 45(18): 7799-7803.

## **P111: Spatial localisation and changes in distribution of FtsH proteases during light stress and nutrient deprivation in *Synechocystis* sp. PCC 6803**

**Joanna Sacharz<sup>1</sup>, Nigel J Burroughs<sup>2</sup>, Samantha J Bryan<sup>1</sup>, Edward M Spence<sup>3</sup>, and Conrad W Mullineaux<sup>1</sup>**

<sup>1</sup>School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, United Kingdom; <sup>2</sup>Systems Biology Centre, Coventry House, University of Warwick, Coventry CV4 7AL, United Kingdom; <sup>3</sup>Department of Pharmaceutical Sciences, King's College London, SE1 9NH, United Kingdom

Protein quality control and proteolysis are involved in cell maintenance and environmental acclimation in both prokaryotes and eukaryotes. The AAA proteases are a conserved class of ATP-dependent proteases that mediate the degradation of membrane proteins in bacteria, mitochondria and chloroplasts [1]. The cyanobacterium *Synechocystis* sp. PCC 6803 has four membrane-bound ATP-dependent FtsH proteases: FtsH1, FtsH2, FtsH3 and FtsH4. The roles of FtsH1, FtsH3 and FtsH4 are not clear [2] however FtsH2 has been shown to play an important role in many physiological processes including selective degradation of the D1 protein during Photosystem II repair [3]. To better understand the Photosystem II repair mechanism and the roles of FtsH proteases, we characterised the localisation of the four FtsH proteases by visualising the proteins *in vivo* in live cyanobacterial cells using fusion with enhanced green fluorescent protein (eGFP) combined with confocal microscopy. We compared localisation under normal conditions and stresses such as high light in which the FtsH proteases are supposed to be more active. We show FtsH2, FtsH3 and FtsH4 are found in the thylakoid membrane while FtsH1 is located in the plasma membrane. We also show that all the FtsH proteins are capable of forming discrete spots which may suggest that protein turnover, particularly the turnover of D1, is localised to specific areas in the thylakoid and plasma membrane. These areas may resemble proteasomes, the protein degradative machines found in the nucleus and cytoplasm of eukaryotic cells.

[1] Langer T (2000). AAA proteases: cellular machines for degrading membrane proteins. *Trends Biochem Sci.* 25:247-51. Review; [2] Mann NH, Novac N, Mullineaux CW, Newman J, Bailey S, Robinson C (2000). Involvement of an FtsH homologue in the assembly of functional photosystem I in the cyanobacterium *Synechocystis* sp. PCC 6803. *FEBS Lett.*;479:72-7; [3] Silva P, Thompson E, Bailey S, Kruse O, Mullineaux CW, Robinson C, Mann NH, Nixon PJ (2003). FtsH is involved in the early stages of repair of photosystem II in *Synechocystis* sp PCC 6803. *Plant Cell.* 15:2152-64



## P112: Expanding the Direct HetR Regulon in *Anabaena* sp. strain PCC 7120

**Patrick Videau<sup>1</sup>, Erik A. Feldmann<sup>2</sup>, Shuisong Ni<sup>2</sup>, Michael A. Kennedy<sup>2</sup>, and Sean M. Callahan<sup>1</sup>**

<sup>1</sup>Department of Microbiology, University of Hawaii at Manoa, Honolulu, HI, USA; <sup>2</sup>Department of Chemistry and Biochemistry, Miami University, Oxford, Ohio, USA

*Anabaena* sp. strain PCC 7120 is a cyanobacterium that differentiates specialized heterocyst cells to fix atmospheric dinitrogen in response to nitrogen limiting conditions. The master regulator of differentiation, HetR, exerts a global effect on heterocyst morphogenesis and function, possibly through the binding of DNA. HetR is known to bind within the promoter regions of 5 genes [1-3]; *hetP*, *hetR*, *hetZ*, *patS*, and *hepA*. Here, we present evidence for HetR binding to 168 potential targets throughout the chromosome.

Initially, three additional 29-bp HetR binding sites upstream of the *hetZ*, *trpE*, and *hepA* genes were identified based on similarity to the 17-bp inverted repeat (IR) located upstream of the *hetP* gene. A search of the *Anabaena* genome for sites similar to the *hepA*-like IR revealed 168 potential HetR binding sites. 155 sites are located in predicted intergenic regions and 100 sites are paired, with the two IRs separated by 13 bp. The locations of binding sites relative to published transcriptional start sites suggests both activating and inhibitory roles for HetR [4].

Phylogenetic trees were constructed for single and paired sites; two sites were chosen for study from each of the two main branches of each tree. The eight selected single and paired sites, represented by 29 bp and 59 bp DNA fragments, respectively, each displayed mobility shifts during EMSA experiments, indicative of HetR binding. A transcriptional fusion to green fluorescent protein (GFP) with the *hepA* promoter showed fluorescence localized to heterocyst cells. Following mutation of the 17-bp IR HetR binding site in the *hepA* transcriptional fusion vector, heterocyst-specific fluorescence was abolished, consistent with binding to this site to activate transcription from this promoter. Transcriptional fusions with the promoters of the eight representative genes to GFP revealed some heterocyst-specific, HetR-dependent fluorescence. Mutation of all eight genes located downstream of the representative HetR binding sites will be assessed for the ability to form a developmental pattern and differentiate mature, nitrogen-fixing heterocysts. This work suggests that HetR directly regulates the transcription of a large number of genes related to heterocyst differentiation.

[1] Huang, X. *et al.* (2004) PNAS. 101:4848-4853; [2] Higa, K. C. and S. M. Callahan (2010) Mol Microbiol. 77:562-574; [3] Du, Y. *et al.* (2012) J Bact. 194:2297-2306; [4] Mitschke, J. *et al.* (2011) PNAS. 108:20130-20135

## P113: Metabolic engineering of *Synechocystis* sp. PCC6803 for biofuel production

**Philipp Savakis, S. Andreas Angermayr, Alejandra de Almeida, Klaas J. Hellingwerf**

Molecular Microbial Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam

Carbon dioxide has been discussed to contribute to the greenhouse effect of the earth's atmosphere. It is, however, also an abundant C<sub>1</sub>-building block that photosynthetic organisms utilize in the Calvin-Benson cycle to generate organic matter. The reducing equivalents required to fix CO<sub>2</sub> are supplied as NADPH created via oxygenic photosynthesis. Traditional approaches for biofuel synthesis involve the generation of biomass by photoautotrophic organisms and –after harvesting– its subsequent conversion to fuel compounds using fermentative organisms. These distinct processes can be combined to yield a single 'photo-fermentative' organism [1]. Our research focuses on the construction of such organisms. We aim to construct heterologous fermentative pathways and to introduce these into the unicellular cyanobacterium *Synechocystis* sp. PCC6803. The resulting *Synechocystis* strains could harness photon energy to synthesize biofuel directly from carbon dioxide, with oxygen being the only by-product formed, particularly when the background metabolism of the organism is optimized to accommodate the heterologous pathway.

[1] K. J. Hellingwerf, M. J. Teixeira de Mattos, J. Biotechnol. 2009, 142(1), 87-90.



## P114: Overexpression of an RNA polymerase sigma factor SigE increases bioplastic production in cyanobacteria

**Takashi Osanai<sup>1,2</sup>, Keiji Numata<sup>3</sup>, Akira Oikawa<sup>1</sup>, Kiminori Toyooka<sup>1</sup>, Mayuko Sato<sup>1</sup>, Ayuko Kuwahara<sup>1</sup>, Hiroko Iijima<sup>1</sup>, Yoshiharu Doi<sup>3</sup>, Kan Tanaka<sup>4</sup>, Kazuki Saito<sup>1,5</sup>, and Masami Yokota Hirai<sup>1</sup>**

<sup>1</sup>Plant Science Center, RIKEN, Kanagawa, Japan; <sup>2</sup>JST, PRESTO, Tokyo, Japan; <sup>3</sup>Biomass Engineering Program, RIKEN, Saitama, Japan; <sup>4</sup>Chemical Resources Laboratory Tokyo Institute of Technology, Kanagawa, Japan; <sup>5</sup>Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan

Bioplastic production by cyanobacteria has received increasing attention because of direct use of CO<sub>2</sub> and light energy. Here we show increased production of polyhydroxybutyrate (PHB), one of biodegradable polyesters, by overexpressing a sigma factor SigE in *Synechocystis* sp. PCC 6803. Overexpression of SigE resulted in increased protein levels related to glycogen catabolism, the oxidative pentose phosphate pathway, and polyhydroxyalkanoate (PHA) biosynthesis. Accumulation of PHB is enhanced by SigE overexpression under nitrogen-limited conditions, whereas the molecular weights of PHBs were similar between the parental wild-type and SigE overexpression strain. Phenotypic analysis demonstrated that cells overexpressing SigE were precipitated faster compared to wild-type cells due to the increased cell sizes. Although gene expressions in response to nitrogen starvation were disturbed by SigE overexpression, these results indicate that genetic engineering of a transcriptional regulator is useful for PHB production in unicellular cyanobacteria.

1. Osanai et al. J. Biol. Chem. 286, 30962-30971, 2011; 2. Osanai et al. Proc. Natl. Acad. Sci. USA 106, 6860-6865, 2009; 3. Osanai et al. J. Biol. Chem. 280, 30653-30659, 2005.

## P115: Characterization of nickel transport in *Synechocystis* sp. PCC 6803

**Dörte Hoffmann<sup>1</sup>, Kirstin Gutekunst<sup>1</sup>, Rüdiger Schulz<sup>1</sup>, and Jens Appel<sup>1,2</sup>**

<sup>1</sup>Botanisches Institut, Universität Kiel, Am Botanischen Garten 1-9, 24118 Kiel, Germany; <sup>2</sup>School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Nickel is an important trace element. It is part of the active site of carbon monoxide dehydrogenases, ureases, superoxide dismutases (SOD), methyl-CoM reductases, glyoxylases I, acireductone dioxygenases, and NiFe-hydrogenases. Cyanobacteria are known to express urease, nickel specific SOD, and NiFe-hydrogenases.

We could show that increasing magnesium concentrations inhibit the uptake of nickel in cells of *Synechocystis* sp. PCC 6803. These results suggest that in the range of  $\mu\text{M}$  concentrations Ni<sup>2+</sup> is taken up nonspecifically via magnesium transporters. However, the usually low availability of nickel (3-9 nM) in aqueous habitats and the accumulation of nickel inside *Synechocystis* calls for a high affinity nickel-transporter. This is especially true for marine cyanobacteria living in iron scarce environments harboring only the nickel specific type of the superoxide dismutase [1].

The deletion of a putative candidate gene, *hupE* (*slr2135*), only abolished cobalt uptake in *Synechocystis* whereas the activity of nickel enzymes such as the NiFe-hydrogenase or the urease was unaffected [2].

Subsequent in-silico analysis of prokaryotic genomes suggested the presence of a Ni<sup>2+</sup>/Co<sup>2+</sup> specific ABC-transporter in the genome of a number of cyanobacteria [3]. The investigation of a respective deletion mutant revealed that its growth was severely affected at low nickel concentrations. Moreover, this mutant had only very low activities of the NiFe-hydrogenase and the urease. Measurements of nickel uptake affinity and their implications regarding the specificity of the transporter are discussed.

[1] Dupont CL, Barbeau K, Palenik B (2008) Ni uptake and limitation in marine *Synechococcus* strains, Appl Environ Microbiol 74:23-31; [2] Hoffmann D, Gutekunst K, Klissenbauer M, Schulz-Friedrich R, Appel J (2006) Mutagenesis of hydrogenase accessory genes of *Synechocystis* sp. PCC 6803 – additional homologues of *hypA* and *hypB* are not active in hydrogenase maturation, FEBS J 273:4516-4527; [3] Rodionov DA, Hebbeln P, Gelfand MS, Eitinger T (2006) Comparative and functional genomic analysis of prokaryotic nickel and cobalt transporters: evidence for a novel group of ATP-binding cassette transporters, J Bacteriol 188:317-327.

## P116: Regulatory Mechanisms of CrtJ, an Aerobic Repressor of Photosystem Gene Expression in *Rhodobacter capsulatus*

Zhuo Cheng, Jiang Wu, Karan Li and Carl E. Bauer

Department of Molecular and Cellular Biochemistry, Indiana University, Bloomington, Indiana, USA

*Rhodobacter capsulatus* is a model organism for studying the physiology of purple non-sulfur photosynthetic bacteria, a group whose metabolic versatility enables them to thrive in wide range of environments. As photosynthetic organisms these bacteria must tightly regulate biosynthesis of photosystem components, which are essential during anaerobic photosynthetic growth, but unnecessary under aerobic conditions. The protein CrtJ in *R. capsulatus* is a major regulator of genes coding for enzymes involved in the biosynthesis of heme, bacteriochlorophyll and carotenoids as well as structural proteins of light harvesting-II complex [1]. Previous studies have demonstrated that CrtJ binds promoters containing two conserved palindromes (TG<sub>12</sub>ACA), and that redox regulated binding is dependent on the formation of an intramolecular disulfide bond between two redox active cysteines (C249 and C420) [2]. However, we recently demonstrated that Cys420 can form additional redox active derivatives such as a cysteine sulfenic acid (-SOH) modification and that the presence of a hydroxyl group stimulates the DNA binding activity of CrtJ. We also show that the hydroxyl group (-OH) of Ser can mimic cysteine sulfenic acid (-SOH) as a Cys420 mutation to Ser results in a constitutive active form of CrtJ when assayed *in vitro* and *in vivo*. These results provide a molecular mechanism whereby different oxidative modifications of a redox active Cys420, which is located in the DNA binding motif of CrtJ, leads to differential effects on tetrapyrrole gene expression.

In addition to redox control, we also show that CrtJ is regulated by an antirepressor called AerR that utilizes the tetrapyrrole cobalamine (B<sub>12</sub>) as a cofactor. In many proteobacteria, *crtJ* homologs are immediately preceded by *aerR* indicating a potential interaction between the two proteins. Pull down and Microscale Thermophoresis assays demonstrate that there is indeed an interaction between CrtJ and AerR. We also demonstrate that purified AerR contains hydroxo-B<sub>12</sub> (OH-B<sub>12</sub>) bound at a conserved histidine (His145). Photosystem synthesis in a *R. capsulatus* strain containing an AerR H145A mutation no longer responds to alterations in light intensity indicating that cobalamin has important regulatory role in photosystem synthesis.

[1] Ponnampalam, S. N.; Buggy, J. J.; Bauer, C. E., *J Bacteriol* 1995, 177 (11), 2990-7; [2] Masuda, S.; Dong, C.; Swern, D.; Setterdahl, A. T.; Knaff, D. B.; Bauer, C. E., *Proc Natl Acad Sci U S A* 2002, 99 (10), 7078-83; [3] Leonard, S. E.; Reddie, K. G.; Carroll, K. S., *ACS Chem Biol* 2009, 4 (9), 783-99.

## P117: Perforation of the septal murein by cell wall amidase AmiC is essential for cell-cell communication and cell differentiation in filamentous cyanobacteria of the order Nostocales

Josef Lehner, Susanne Berendt, Karl Forchhammer, Iris Maldener

IMIT, Organismic Interactions, University of Tübingen, Auf der Morgenstelle 28, 72076 Tübingen, Germany; iris.maldener@uni-tuebingen.de

Filamentous cyanobacteria of the order Nostocales show differentiation of single cells within the filament - a key characteristic of multicellularity. These cells, the so-called heterocysts, are specialized for the fixation of atmospheric N<sub>2</sub> and provide the entire filament with nitrogen fixation products. Heterocyst differentiation, involves modulation of the cell wall, obvious by the deposition of an extra envelope upon the Gram-negative cell wall and the altered cell shape. Cell wall hydrolyzing enzymes, like amidases, were shown to be involved in peptidoglycan remodeling in Gram-negative bacteria. We could recently show that a AmiC-type amidase (NpF1846) is required for normal filament morphology and cell differentiation in *Nostoc punctiforme* ATCC 29133 [1]. Here, we present the functional characterization of a homologue *amiC*-gene from *Anabaena* sp. strain PCC7120. The mutant in *amiC1* (*aln0092*) was not able to differentiate heterocysts, and hence could not grow on N<sub>2</sub> as sole nitrogen source. Using translational GFP-fusions, we could show that the AmiC-protein was predominantly present in the septa of freshly divided cells and at the polar neck of maturing heterocysts. Furthermore, the *amiC1* deficient mutant is impaired in septum-dependent cell-cell communication as revealed by FRAP analysis. To further characterize the function of AmiC in cell septum maturation, peptidoglycan sacculi were prepared from wild-type and mutant cells and analyzed by electron microscopy. The peptidoglycan sacculus is continuous along the filament and contains disc-shaped septal peptidoglycan structures. This septal discs show an array of perforations with 20 nm in diameter in the wild type but not in the *amiC1* mutant. *In vivo* cell-wall staining of filaments with fluorescently labeled vancomycin corroborates these results and reveals distinct disc-shaped septal peptidoglycan, whose maturation depends on AmiC. Together, our results reveal a new role for cell wall amidases in specifically perforating the septal murein for the formation of cell-cell junction pores and by this establishing a multicellular organism.

[1] Lehner J, Zhang Y, Berendt S, Rasse TM, Forchhammer K, Maldener I. (2011) The morphogene AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme*. *Mol Microbiol.* 79:1655-1669.

## P118: Transition metals and phototrophic growth

**Adrian Kölsch<sup>1</sup>, Marco Rügen<sup>2</sup>, Ralf Steuer<sup>2</sup>, Nir Keren<sup>3</sup>, Ilka Axmann<sup>2</sup>**

<sup>1</sup>Institute for Biology, Humboldt University, Berlin, Germany, <sup>2</sup>Institute for Theoretical Biology, Humboldt University, Berlin, Germany; <sup>3</sup>Dept. of Plant & Environmental Science, The Hebrew University of Jerusalem, Israel

The Cyanobacterium *Synechocystis* sp. PCC 6803 is a freshwater cyanobacterium which has been studied intensely in the past [1]. Like many other cyanobacterial species, it can grow either autotrophically or heterotrophically, and regulate its metabolism by a circadian clock. Growth of cyanobacteria has been accurately estimated by mathematical models using the flux of macronutrients to estimate maximal biomass formation [2]. So far the bioavailability of transition metals has not been considered as a constraint in computational flux prediction.

In photosynthetic organisms large amounts of transition metals are necessary for the photosynthetic apparatus, e.g. cyanobacteria have a 10 fold higher iron quota than non-photosynthetic organisms. Nevertheless many habitats, including vast regions of the planets oceans, are characterized by low concentrations of trace metals, leading to a limited activity of key-metalloenzymes. Many of these metalloenzymes take part in aquisition of major nutrients, and hence can restrict the growth of organisms.

To overcome metal restricted growth conditions cyanobacteria use differential gene regulation, such as expression of CP43' (isiA), which increases the number of chlorophylls at photosystem I under iron limiting conditions [3], or using flavodoxin instead of the iron sulfur protein ferredoxin [4].

Our aim is to characterize the growth of the cyanobacterium *Synechocystis* sp. PCC 6803, in the context of its circadian clock, under metal replete and deficient conditions and characterize metal dependent proteins. Our results will be integrated into a flux-balance analysis, to improve the predictions of metabolic adaption. This will help us to gain knowledge about cyanobacteria as a natural resource, and may be a helpful tool for biotechnological applications, and possibly even increase biofuel productivity.

[1] Kaneko T., Sato S., Kotani H., Tanaka A., Asamizu E., Nakamura Y., Miyajima N., Hirose M., Sugiura M., Sasamoto S., Kimura T., Hosouchi T., Matsuno A., Muraki A., Nakazaki N., Naruo K., Okumura S., Shimpo S., Takeuchi C., Wada T., Watanabe A., Yamada M., Yasuda M., Tabata S., 1996, DNA Research, 3, 109-136; [2] Knoop H., Zilliges Y., Lockau W., Steuer R., 2010, Plant Physiology, 154, 410-422; [3] Nield J., Morris E.P., Bibby T.S., Barber J., 2003, Biochemistry, 42, 3180-3188; [4] Mayhew S.E. and Ludwig M.L., 1975, The Enzymes, 12, 57-118.

## P119: Structure-function relationships of the rhodopin 3,4-desaturase (CrtD) of *Rhodospirillum rubrum*

**Caroline Autenrieth<sup>1</sup>, and Robin Ghosh<sup>1</sup>**

<sup>1</sup>Department of Bioenergetics, Biological Institute, University of Stuttgart, Stuttgart, Germany

It is now well established that the carotenoid present in the light-harvesting (LH) 1 complex is in the *all-trans* configuration. It has also been demonstrated that *in situ* modification of the carotenoid "ends" i.e. the 3,4,3',4'-dehydrogenation, hydroxylation and methoxylation can occur during LH1 maturation. For each of these carotenoid maturation steps mentioned above, only a single enzyme (CrtD, CrtC, and CrtF, respectively) is present, which from hydropathy analysis appear to be localized to the cytoplasmic side of the membrane. This raises the interesting question as to how maturation of an *all-trans* carotenoid can occur *in situ*. In this study we focus on the *crtD* gene to cast light upon this question. The *crtD* gene, encoding the rhodopin 3,4,3',4'-desaturase of *Rhodospirillum rubrum*, was cloned into a pRK404-derived broad host range vector and then subjected to extensive random mutagenesis. The mutagenized vectors were then used to complement a *crtD* deletion mutant (*R. rubrum* strain ST4 [1]). The resulting complemented mutants yielded colonies showing a variety of different colours (from brown to pink to purple), which were isolated for subsequent carotenoid content analysis. DNA sequencing of the interesting mutants yielded insights into the nature of the active site of CrtD. These results will be presented here.

[1] Komori, M., R. Ghosh, S. Takaichi, Y. Hu, T. Mizoguchi, Y. Koyama, and M. Kuki. 1998. A null lesion in the rhodopin 3,4-desaturase of *Rhodospirillum rubrum* unmasks a cryptic branch of the carotenoid biosynthetic pathway. Biochemistry. 37:8987-8994.

## P120: A chimeric protein Pix0522 reveals the function of the BLUF domain C-terminal $\alpha$ -helices for light-signal transduction

Shukun Ren<sup>1</sup>, Koji Hasegawa<sup>2</sup>, Hiroyuki Ohta<sup>1</sup> and Shinji Masuda<sup>1,3</sup>

<sup>1</sup>Center of Biological Resources and Informatics, Tokyo Institute of Technology, Yokohama, Japan; <sup>2</sup>Advancesoft Corporation, Akasaka, Tokyo, Japan; <sup>3</sup>PRESTO, Japan Science and Technology Agency, Saitama, Japan

Blue-light-using flavin (BLUF) proteins from a subfamily of blue-light photoreceptors, are found in many bacteria and algae, and are further classified according to their structures. One type controls light-dependent protein-protein interaction with a downstream factor as shown for PixD, a BLUF photoreceptor found in the cyanobacterium *Synechocystis* sp. PCC6803; another type controls enzyme activity as shown in PapB, a BLUF protein found in the purple bacterium *Rhodospseudomonas palustris*. For the first type of BLUF proteins, the central axes of its two C-terminal  $\alpha$ -helices are perpendicular to the  $\beta$ -sheet of its N-terminal BLUF domain; for the second type of BLUF proteins, the central axes of its two C-terminal  $\alpha$ -helices are parallel to its BLUF-domain  $\beta$ -sheet. In this study, we constructed a chimeric protein, Pix0522, which consists of the core of the PixD BLUF domain and the C-terminal region of PapB, including the two  $\alpha$ -helices, and its light signal transduction were analyzed. Fourier transform infrared spectroscopy detected similar light-induced conformational changes in the C-terminal  $\alpha$ -helices of Pix0522 and PapB. Pix0522 interacts with and activates the PapB-interacting enzyme, PapA, demonstrating the functionality of Pix0522. These results provide direct evidence that the BLUF C-terminal  $\alpha$ -helices function as an intermediary that accepts the flavin-sensed blue-light signal and transmits it downstream during photo-transduction. The result also showed that the chimera protein could interact with the PixD-interacting protein PixE, but the interaction is not sensitive to light. These observations suggest that PixE interacts with the PixD N-terminal domain, and the PixD C-terminal site controls the protein-protein interaction with PixE in the light-dependent manner. The mechanism of light-signal transduction between PixD-PixE will be also discussed.

## P121: Making membranes - composition of the different membrane systems in *Synechocystis* sp. PCC6803

Tiago Toscano Selão<sup>1</sup>, Lifang Zhang<sup>1</sup>, Zoltán Gombos<sup>2</sup>, Eva Selstam<sup>3</sup>, Åke Wieslander<sup>4</sup> and Birgitta Norling<sup>1</sup>

<sup>1</sup>School of Biological Sciences, Nanyang Technological University, Singapore; <sup>2</sup>Biological Research Center, Szeged University, Szeged, Hungary; <sup>3</sup>Umeå Plant Science Center, Umeå, Sweden; <sup>4</sup>Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

*Synechocystis* sp. PCC 6803 is widely used as a model organism for the study of photosynthesis and respiration. However, many of the details behind genesis of the membrane systems in these cells still remain unknown. Two opposing models have been presented regarding the internal membrane organization, one in which the thylakoid membrane forms a separate system and another in which the thylakoid membrane connects with the plasma membrane, be it in a temporary or permanent fashion.

In order to further understand membrane biogenesis, we have used a purification method combining sucrose gradients and two-phase partitioning. This method yields two different plasma membrane fractions, a "lighter" fraction with higher lipid content (PM1) and a "heavier" fraction, with a higher protein-to-lipid ratio (PM2), as well as a purified thylakoid fraction (TM). We have studied the lipid, fatty acid and pigment composition in the different fractions, as well as the activity of the enzyme responsible for the synthesis of the main lipid class, monoglucosyldiacylglycerol synthase. There are subtle differences between both kinds of plasma membranes and between these and the thylakoid membrane in what regards the fatty acid content. However, the greatest difference resides in the pigment composition, with the "light" PM1 having a much higher carotenoid content, in comparison to the "heavy" PM2.

We have also studied the photodamage-induced repair of D1 using pulse-chase in the separate membrane classes. Recently, we have localized the insertase YidC exclusively in the TM fraction. Our data suggests that pD1 is inserted in the thylakoid membrane and its processing takes place either in the thylakoid membrane or in a subfraction thereof.

## P122: Insights into an essential two component system in the obligate photoautotroph *Synechococcus elongatus* PCC 7942

Javier Espinosa, Félix Moronta-Barrios and Asunción Contreras

División de Genética, Universidad de Alicante, Spain

The NblS-RpaB signaling pathway, the most conserved two-component system in cyanobacteria, regulates photosynthesis and acclimatization to a variety of environmental conditions such as high light. Although the system is essential for growth under standard non stress conditions, suggesting the implication of the signaling system in cellular homeostasis little is known on the nature of the relevant essential processes. The response regulator RpaB is regulated by specific (de)phosphorylation from the histidine kinase NblS. RpaB and its phosphorylatable residue Asp56 are both required for viability of *Synechococcus elongatus* PCC 7942. The relatively high stability of RpaB-P allowed us, to show *in vivo* changes in the ratio of phosphorylated to non-phosphorylated forms of a response regulator. The phosphorylated form of RpaB is present in *Synechococcus elongatus* PCC 7942 cells growing under standard laboratory conditions and high light stress affected the ratio of phosphorylated to non-phosphorylated RpaB.

To gain information on the RpaB role under non stress conditions we now study the *in vivo* impact of relatively mild and genetically stable alterations at the *rpaB* gene of *Synechococcus elongatus* PCC 7942. We found that altering the total levels of RpaB and/or interfering with phosphorylation of the protein led to morphological defects.

## P123: Characterization of the extracellular matrix proteins in *Nostoc cyanobacteria*

**Kaori Inoue-Sakamoto<sup>1,\*</sup>, Takumi Natsume<sup>2</sup>, Masaaki Kanamori<sup>2</sup>, Tomoya Asano<sup>3</sup>, Takumi Nishiuchi<sup>3</sup>, and Toshio Sakamoto<sup>2</sup>**

<sup>1</sup>Department of Applied Bioscience, College of Bioscience and Chemistry, Kanazawa Institute of Technology. Nonouchi 921-8501, Japan; <sup>2</sup>School of Natural System, College of Science and Engineering, Kanazawa University. Kakuma, Kanazawa 920-1192, Japan; <sup>3</sup>Division of Functional Genomics, Advanced Science Research Center, Kanazawa University. Takara-machi, Kanazawa 920-0934, Japan; \*E-mail: ksakamot@neptune.kanazawa-it.ac.jp

*Nostoc* is a group of nitrogen-fixing cyanobacteria found in a variety of environments and often forms colonies composed of filaments of moniliform cells in jellylike sheath. The terrestrial cyanobacterium *Nostoc commune* has a marked capacity to tolerate simultaneous stresses of desiccation, UV irradiation, extreme temperatures and oxidation, and can survive long-term in a desiccated state. *N. commune* forms visible colonies in which cellular filaments embed within extracellular matrices (ECM) [1]. The major component of ECM is extracellular polysaccharides (EPS), accounting for 80% by weight of desiccated *N. commune* colonies, and it is thought that cyanobacterial EPS play a pivotal role in protecting cells from various stresses in severe environments. The major protein component in *N. commune* ECM is a water stress protein (WspA) [2]. More than 70% of the total soluble proteins in desiccated *N. commune* colonies are WspA and its relatives, and synthesis and secretion of WspA were induced by desiccation or UV irradiation [1, 2]. The aquatic *Nostoc verrucosum* forms macroscopic colonies, which consist of cellular filaments and ECM materials. In addition, WspA was present in *N. verrucosum* as a major soluble protein component, suggesting that the production of ECM and WspA are not directly linked to extreme desiccation tolerance in *N. commune* [3].

In this study, the ECM proteins in *N. commune* were investigated to elucidate the components and/or mechanisms required for tolerance against multiple stresses. The ECM proteins were extracted from *N. commune* colonies and separated by 2-D PAGE. In addition to WspA, several proteins were detected by CBB staining. The protein spots were digested by trypsin and the sequences of the peptides were determined by MALDI-TOF-MS. Antioxidant proteins such as iron superoxide dismutase (SOD) and catalase, and iron reservoir protein ferritin were identified. The extract from dry *N. commune* colonies contained SOD activity, and the activities were detected in cell extract as well as in ECM fraction by activity staining. These results suggest that the ECM contains enzymes for protecting cells from oxidative stress in *N. commune*. The ECM proteins of other *Nostoc* species will be presented.

[1] Wright, D.J., Smith, S.C., Joardar, V., Scherer, S., Jervis, J., Warren, A., Helm, R.F. and Potts, M. (2005) *J. Biol., Chem.* 280: 4-271-40281; [2] Scherer, S. and Potts, M. (1989) *J. Biol., Chem.* 264: 12546-12553; [3] Sakamoto, T., Kumihashi, K., Kunita, S., Masaura, K., Inoue-Sakamoto, K. and Yamaguchi, M. (2011) *FEMS Microbiol. Ecol.* 77:385-394.

## P124: Mycosporine like amino acid biosynthesis in *Anabaena variabilis*

**Edward Spence<sup>1</sup>, Walt Dunlap<sup>2</sup>, Malcolm Shick<sup>3</sup> and Paul Long<sup>1</sup>**

<sup>1</sup>Institute of Pharmaceutical Science and Department of Chemistry, King's College London, 50 Stamford Street, London SE1 9NH, United Kingdom; <sup>2</sup>Centre for Marine Microbiology and Genetics, Australian Institute of Marine Science, Townsville, Queensland 4810, Australia; <sup>3</sup>School of Marine Sciences, University of Maine, 5751 Murray Hall, Orono, ME 04469-5751 U.S.A.

**Introduction:** Cyanobacteria are phototrophic prokaryotes that have evolved ultraviolet-screening mycosporine-like amino acids (MAAs) to palliate harmful effects of their obligatory exposure to solar radiation. Evidence suggesting that MAA biosynthesis derives from the shikimic acid pathway was first provided by specific uptake of the shikimate intermediate [U-<sup>14</sup>C] 3-dehydroquinic acid by the plant pathogen *Trichothecium roseum*, to give the structurally related fungal mycosporines. Similarly, <sup>14</sup>C-pyruvate via phosphoenol pyruvate, an obligate precursor of the shikimate pathway, yielded radiolabelled MAAs in the cyanobacterium *Chlorogloeopsis* sp. PCC 6912, whereas <sup>14</sup>C-acetate (polyketide pathway) did not. Recently, however, unambiguous elucidation of the MAA biosynthetic pathway in cyanobacteria was reported (1). Biosynthesis of mycosporine-glycine and shinorine in *Anabaena variabilis* and *Nostoc punctiforme* proceeded from the intermediate sedoheptulose 7-phosphate (SH 7-P) of the pentose phosphate pathway, rather than 3-deoxy-D-arabinoheptulosinate 7-phosphate (DAHP) of the shikimate pathway. The cyanobacterial SH 7-P cyclase, 2-*epi*-5-*epi*-valiolone synthase (EVS), is strikingly similar to the DAHP cyclase, 3-dehydroquininate synthase, of the shikimate pathway, both being of the same superfamily of enzymes leading to the expected MAA precursor, 4-deoxygadusol. Genome mining has revealed EVS to be prevalent in many cyanobacteria known to produce MAAs, and accordingly the gene encoding this enzyme is typically absent in non-producers.

**Methods:** An in-frame deletion of the EVS homologue (*Ava\_3858*) was generated and characterised. Complete segregation of the mutant was only achieved with the addition of fructose.

**Results:** Deletion of the EVS homologue in *A. variabilis* does not affect UV induced MAA biosynthesis (2). However, the EVS mutant had a notable decrease in the phycocyanin content, which was exacerbated in the presence of fructose. Morphological changes observed when wild type cells are grown with fructose were not observed with the EVS mutant.

**Conclusion:** We conclude that functionally duplicate, yet distinct convergent pathways for the biosynthesis of MAAs in *Anabaena* exist, involving both the pentose phosphate pathway and the shikimic acid pathway. The significant loss of phycocyanin in the EVS mutant lead us to hypothesize that the calvin cycle intermediate sedoheptulose 7-phosphate and EVS homologue *Ava\_3858*, form the basis for the control of photosynthetic flux through the accumulation of sedoheptulose 7-phosphate, which triggers the degradation of the phycobilisome via an unidentified mechanism.

1. E. P. Balskus, C. T. Walsh, The genetic and molecular basis for sunscreen biosynthesis in cyanobacteria. *Science* 329, 1653 (Sep, 2010); 2. E. Spence, W. C. Dunlap, J. M. Shick, P. F. Long, Redundant pathways of sunscreen biosynthesis in a cyanobacterium. *Chembiochem* 13, 531 (Mar, 2012).





# **POSTER>SESSION IV**

## **BIOENERGETICS, PROTEOMICS AND GENOMICS**



## POSTERS > IV. BIOENERGETICS, PROTEOMICS AND GENOMICS

### P125: Selection of Suitable Reference Genes for RT-qPCR Analyses in Cyanobacteria

Filipe Pinto<sup>1,2</sup>, Catarina C Pacheco<sup>1</sup>, Daniela Ferreira<sup>1,2§</sup>, Pedro Moradas-Ferreira<sup>1,3</sup>, Paula Tamagnini<sup>1,2</sup>

<sup>1</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; <sup>2</sup>Faculdade de Ciências, Departamento de Biologia, Universidade do Porto, Porto, Portugal; <sup>3</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal; <sup>§</sup>Current address: Department of Microbiology, University of California, Davis, CA, USA.

Cyanobacteria are a group of photosynthetic prokaryotes that have a diverse morphology, minimal nutritional requirements and metabolic plasticity that has made them attractive organisms to use in biotechnological applications. The use of these organisms as cell factories requires the knowledge of their physiology and metabolism at a systems level. For the quantification of gene transcripts real-time quantitative polymerase chain reaction (RT-qPCR) is the standard technique. However, to obtain reliable RT-qPCR results the use and validation of reference genes is mandatory. Towards this goal we have selected and analyzed twelve candidate reference genes from three morphologically distinct cyanobacteria grown under routinely used laboratory conditions. The six genes exhibiting less variation in each organism were evaluated in terms of their expression stability using geNorm, NormFinder and BestKeeper. In addition, the minimum number of reference genes required for normalization was determined. Based on the three algorithms, we provide a list of genes for cyanobacterial RT-qPCR data normalization. To our knowledge, this is the first work on the validation of reference genes for cyanobacteria constituting a valuable starting point for future works [1].

[1] Pinto F, Pacheco CC, Ferreira D, Moradas-Ferreira P and Tamagnini P (2012) Selection of suitable reference genes for RT-qPCR analyses in cyanobacteria. PLoS One 7(4): e34983.

### P126: Functional genomic of the response to arsenic by the cyanobacterium *Synechocystis* sp. PCC 6803

Ana M<sup>a</sup> Sánchez-Riego, Luis López-Maury and Francisco J. Florencio

Instituto de Bioquímica Vegetal y Fotosíntesis. Universidad de Sevilla-CSIC. Avenida Américo Vespucio 49, 41092-Sevilla. SPAIN.

Arsenic is one of the most important global environmental pollutants which displays a variety of toxic effects on living organisms resulting in severe disturbance of cellular metabolism. Because of the wide use and distribution of arsenic compounds, arsenic resistance is widespread among living organisms. Arsenic resistance in *Synechocystis* sp. PCC 6803 is mediated by *arsBHC* operon, that includes an arsenite transporter gene, *arsB*, an *arsH* homologue without a clear function in arsenic resistance and an arsenate reductase gene, *arsC* [1] that uses the glutathione/glutaredoxin system for reduction. There are two additional genes coding for an arsenate reductase: *arsI1* and *arsI2*, which are nearly identical to each other. *arsBHC* operon is regulated by the *ArsR* repressor (encoded by a gene not linked, *slI1954*). *arsI* genes are expressed at low levels and apparently are not regulated by the presence of arsenic in the environment [2].

In order to characterize the physiological responses to arsenic in *Synechocystis*, we analyzed the changes in gene expression using microarrays. We analyzed the response to arsenite (As<sup>III</sup>) and arsenate (As<sup>V</sup>) in WT strain and the mutants unable to metabolize As<sup>V</sup> (SARS12) or As<sup>III</sup> (SARSB) and in the mutant lacking the repressor *ArsR* (SARSR). Our results indicate that arsenite promotes a general activation of the genes involved in the oxidative stress response, some heavy metal detoxification genes and potassium transport genes, and repress the expression of genes involved in photosynthesis and ATP synthesis. On the other hand, arsenate represses the genes related to carbon assimilation and those related to phosphate transport, due to the analogy between phosphate and arsenate. This response is increased in the mutants SARSB and SARS12, which are hypersensitive to the presence of As<sup>III</sup> and As<sup>V</sup>, respectively, while that is almost absent in the mutant SARSR, which shows high resistance to arsenic. Our results also indicate that *ArsR* control the expression of *arsBHC* operon, as expected, but also the two genes that are downstream to it (*rpaB* and *slr0948*) and the gene that is up-stream of *arsB* but in the opposite strand (*slI0914*).

In order to clarify whether *rpaB* and *slr0948* genes are expressed forming an operon with *arsBHC* operon, we analyzed its expression by northern blot and RT-PCR. Our results indicated that *rpaB* and *slr0948* are expressed as a dicistronic mRNA in control cells and as a polycistronic mRNA (*arsBHCrpaBslr0948*) in response to arsenic treatment. On the other hand, we analyzed the survival of the mutants lacking the *slI0914* gene, compared to WT strain in the presence of As<sup>III</sup>. The results indicated that this protein is not related to arsenic resistance in *Synechocystis*.

This work has been financed by EU funds (FEDER)-BFU2010-15708.

[1] López-Maury, L. et al. (2003). Arsenic sensing and resistance system in the cyanobacterium *Synechocystis* sp. strain PCC 6803. J. Bacteriol., 185 (18):5363-5371.; [2] López-Maury, L. et al. (2009). The glutathione/glutaredoxin system is essential for arsenate reduction in *Synechocystis* sp. strain PCC 6803. J Bacteriol., 191 (11):3534-3543.

## P127: Diversity in Hydrogen Evolution from Bidirectional Hydrogenases in Cyanobacteria from Terrestrial, Freshwater and Marine Intertidal Environments

Ankita Kothari<sup>1</sup> and Ferran Garcia-Pichel<sup>1</sup>

<sup>1</sup>School of Life Sciences, Arizona State University, Tempe, Arizona 85287-4501, USA.

Biohydrogen production from water splitting by oxygenic phototrophs constitutes a conceptually promising avenue because it directly couples the inexhaustible solar energy to the generation of clean biofuel without side products. Cyanobacteria have been deemed as good models for biohydrogen production because of their minimal nutritional requirements and ability to produce hydrogen gas under certain physiological conditions. The major drawbacks of known cyanobacterial platforms for biohydrogen are: i) the overall low yields, ii) the reversibility of the enzymes, and iii) the enzyme's inactivation by oxygen. But, in reality, only a few standard laboratory strains have been examined in this respect. In order to find novel strains with naturally better biohydrogen production traits, we characterized a set of 36 newly isolated cyanobacterial strains isolated from terrestrial, freshwater and marine intertidal settings. We probed for the presence of Ni-Fe hydrogenases, and characterized specific hydrogen evolving activity in the presence of excess reductant. About one half of the strains could produce hydrogen, a trait that corresponded invariably with the detectability of the gene *hoxH*, coding for subunit H in the bidirectional Ni-Fe hydrogenase. Interestingly all our terrestrial isolates were negative for both the gene and its activity. Isolates from marine benthos and freshwater by contrast had a high incidence of positives. Amongst these, we detected two distinctive patterns in hydrogen production. Pattern 1, corresponding to that previously known from *Synechocystis* PCC 6803, encompassed strains whose hydrogenase system produced hydrogen only temporarily, reverting to hydrogen consumption within a short time and after reaching only moderate hydrogen concentrations. Cyanobacteria displaying pattern 2, were obtained from intertidal marine microbial mats, and belonged to *Lyngbya aestuarii* and *Microcoleus chthonoplastes*. They displayed higher rates, did not reverse the direction of the reaction, and, importantly reached much higher concentrations of hydrogen at steady state. These are all very desirable characters for eventual application. Because of their ecological origin, we hypothesized that this increased biohydrogen production capacity in pattern 2 cyanobacteria may have evolved to served a role in the fermentation of photosynthate. In fact, when forced to ferment, pattern 2 cyanobacteria displayed similarly improved characteristics in hydrogen production compared to standard strains. The rates and steady state concentrations of fermentative hydrogen attained in *Lyngbya* strains, but not those of *Microcoleus* strains, could be further enhanced when cultures were pre-acclimated to recurrent nighttime anaerobiosis during growth. Thus intertidal *Lyngbya* strains from microbial mats become of special interest as potential platforms for biohydrogen production research and eventual application.

## P128: Does 6S RNA balance the transcriptional network of cyanobacteria?

Beate Heilmann<sup>1</sup>, Anne Rediger<sup>1</sup>, René Geissen<sup>2</sup>, Rolf Wagner<sup>2</sup> and Ilka M. Axmann<sup>1</sup>

<sup>1</sup>Charité and Humboldt University Berlin, Institute for Biology / Biochemistry, Chausseestr. 117, 10115 Berlin, Germany; <sup>2</sup>Heinrich-Heine University Düsseldorf, Institute of Physical Biology, Universitätsstr. 1, 40225 Düsseldorf, Germany.

6S RNA is indicative of small-non-coding RNAs (sRNAs) which implement complex regulatory networks within diverse bacteria. More than 30 years after sequencing of 6S RNA from *E. coli*, its function as an inhibitor of transcription based on interaction with RNA polymerase holoenzyme (RNAP) during stationary growth, was verified. In recent years, more information about this RNA from other groups of bacteria, like *Bacillus subtilis* and *Legionella pneumophila*, was published. However, information about 6S RNA in cyanobacteria is limited. The homolog of 6S RNA became identified in the freshwater cyanobacteria *Synechococcus elongates* PCC 6301 and *Synechocystis* sp. PCC 6803 as one of the very first cyanobacterial sRNAs. In addition this sRNA was described in different strains from the marine genera *Prochlorococcus* and *Synechococcus* [1].

As a first step towards understanding the function of this small-non-coding RNA in a unicellular cyanobacterium we succeeded to accomplish well described *in vitro* transcription systems of *E. coli* [2]. We observed an interaction of the 6S RNA of *Synechocystis* sp. PCC6803 with RNAP of *E. coli* by gel retardation. The ability to act as a template for a specific *de novo* transcript described for *E. coli* [3] could be replicated with the cyanobacterial sRNA *in vitro*. Furthermore the melting point of the 6S RNA of *Synechocystis* sp. PCC6803 is more than 10 °C lower than determined for *E. coli*.

First results of expression analysis *in vivo* compound a potentially varying behaviour of 6S RNA in cyanobacteria compared to enterobacteria. We could show the accumulation of distinct 6S RNA molecules of different lengths in *Prochlorococcus* MED4 [4]. For the first time we reported the regulation of sRNA levels during growth and the diel cycle. Coordination of biological activities through an internal circadian clock within prokaryotes is confined to cyanobacteria. Therefore, the gene expression system in cyanobacteria, including the functional role of 6S RNA must have adapted accordingly. Advanced physiological characterisation of 6S RNA in cyanobacteria is being investigated with 6S RNA knock-out and overexpression mutants.

(1)Axmann, I.M. et al., Genome Biology, 2005, 6: R73; (2) Rediger, A. et al., 2012, submitted; (3) Geissen, R. et al., RNA Biol., 2010, 7: 564-8; (4) Axmann, I.M. et al., Gene, 2007, 406: 69-78.

## P129: Deletion of *Synechocystis* sp. PCC6803 leader peptidase LepB1 affects photosynthetic complexes and respiration

Lifang Zhang<sup>1</sup>, Tiago Toscana Selao<sup>1</sup>, Siu Kwan Sze<sup>1</sup>, Inger Carlberg<sup>2</sup>, Birgitta Norling<sup>1</sup>

<sup>1</sup>School of Biological Sciences, Nanyang Technological University, Singapore; <sup>2</sup>Department of Biochemistry and Biophysics, Arrhenius Laboratories for Natural Sciences, Stockholm University, Stockholm, Sweden.

The cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis*) has a complex membrane organization with an internal thylakoid membrane in addition to the outer and plasma membranes. This implies the existence of a sophisticated system for the sorting and transport of extracytoplasmic proteins to the different membranes and compartments. Targeting of newly synthesized proteins occurs through the well-characterized Sec and Tat pathways. In contrast to other Gram-negative bacteria all cyanobacteria contain two leader peptidases type 1, LepB1 and LepB2, for processing of sec/tat signals.

In a previous work (1) knockout mutations of the two Leps were studied. It was found, that while LepB2 is totally essential for cell viability, cells with disruption in LepB1 are still able to grow, albeit only under mixed heterotrophic conditions in low light. It was concluded that LepB2 is the major leader peptidase, whereas LepB1 is processing components of the photosynthetic machinery. In the present work we studied the changes in the thylakoid membrane and its protein complexes. We found that the LepB1 deletion mutant ( $\Delta$ LepB1) contained decreased amounts of PSI and cytochrome  $b_6f$  complex, but unchanged amount of PSII. This will give an imbalance of electron flow through the PQ pool, resulting in a PQ over reduction, which explains the light sensitivity of the mutant. To cope with this situation the amount of the antenna, the phycobilisome, decreased in  $\Delta$ LepB1. Furthermore  $\Delta$ LepB1 was found to have twice as much of cytochrome bd oxidase as WT, which results in a larger withdrawal of electrons from PQH<sub>2</sub>. The Chl content was half in  $\Delta$ LepB1 compared to WT, as a consequence of the lowered PSI content. Three of the enzymes involved in Chl biosynthesis showed decreased amount in  $\Delta$ LepB1. The different effects on PSI and cytochrome  $b_6f$  compared to PSII, is suggested to depend on the presence in both PSI and cytochrome  $b_6f$  of an integral membrane protein with a sec-signal, which processing is severely effected in the mutant.

<sup>1</sup>Zhbanko, M. et al. Inactivation of a predicted leader peptidase prevents photoautotrophic growth of *Synechocystis* sp. strain PCC 6803. J. Bacteriol. 2005, 187, (9),3071-8.

## P130: The Gene Expression System in the Green Sulfur Bacterium *Chlorobaculum tepidum* by Conjugative Plasmid Transfer

Chihiro Azai<sup>1,2</sup> and Hirozo Oh-oka<sup>1</sup>

<sup>1</sup>Graduate School of Science, Osaka University, Toyonaka, Japan; <sup>2</sup>Present affiliation: Graduate School of Science, Nagoya University, Nagoya, Japan

Green sulfur bacteria are obligatory anaerobic photoautotrophic bacteria. They have a lot of unique and intriguing features in their photosynthetic system, such as homodimeric structure of the reaction center, an extraordinary large antenna organelle named chlorosome, carbon assimilation by the reductive TCA cycle, and so on. Although these have been studied for many years, they are poorly understood at a molecular level because the strictly anaerobic properties of their components make it difficult to conduct various biochemical studies. Thus molecular genetic analysis is required to advance the research on the green sulfur bacterial photosynthesis. In the early 2000s, the complete genome information and the transformation system based on homologous recombination became available in the mesothermophilic green sulfur bacterium *Chlorobaculum (Cba.) tepidum*. Many knock-out mutants have intensively been isolated and revealed the functions of various genes so far, while the essential genes for photosynthetic growth have barely been subjected to any mutagenic studies because of the photoautotrophy of green sulfur bacteria. Therefore, it has been eagerly desired to develop a gene expression system enabling gene complementation analyses and the protein production for biochemical studies.

In 1995, T.M. Wahlund and M.T. Madigan have reported the plasmid transfer in *Cba. tepidum* by bacterial conjugation with *Escherichia coli* [1], however we could not obtain any transformants which stably harbored plasmids by their reported method. In this study, we tried again to construct an easy-to-use gene expression system using a broad-host-range plasmid, pDSK519 [2]. As a result of reexamination of antibiotic selection markers, we succeeded to introduce the plasmid to *Cba. tepidum* by biparental transfer from *E. coli* S17-1. To construct an expression plasmid in *Cba. tepidum*, we incorporated the 440 bp upstream sequence of the gene encoding the reaction center core-protein into the conjugation plasmid as a promoter for the transcription of an exogenous gene. The availability of this expression plasmid was confirmed by gene complementation experiments, in which the growth defects of  $\Delta$ *cycA* and  $\Delta$ *soxB* mutants were fully rescued by their complementary genes on the plasmids. The protein expression level by the plasmid was also evaluated by introducing the gene encoding the His-tagged reaction center core-protein into the plasmid. The total amount of the functional reaction center complex in the cell was significantly enhanced. Therefore, this gene expression system is highly effective and widely applicable to various kinds of gene transfer experiments in *Cba. tepidum*.

[1]T.M. Wahlund and M.T. Madigan, J. Bacteriol. (1995) 177:2583-2588; [2] N.T. Keen et al., Gene (1988) 70:191-197.

### **P131: Energy transfer in the cyanobacterium, *Synechocystis* sp. PCC 6803, under high-light conditions with different light qualities**

**Erina Kobe<sup>1</sup>, Makio Yokono<sup>2,3</sup>, Shimpei Aikawa<sup>3,4</sup>, Akihiko Kondo<sup>3,4</sup>, and Seiji Akimoto<sup>1,2,3</sup>**

<sup>1</sup>Graduate School of Science, Kobe University, Kobe, Japan; <sup>2</sup>Molecular Photoscience Research Center, Kobe University, Kobe, Japan; <sup>3</sup>JST, CREST, Kobe, Japan; <sup>4</sup>Graduate School of Engineering, Kobe University, Kobe, Japan.

The cyanobacterium *Synechocystis* sp. PCC 6803 has phycobilisomes (PBSs), composed of C-phycocyanin (PC) and allophycocyanin (APC), as peripheral antennae. PCC 6803 is known to have some types of light adaptation to optimize the use of ambient light energy. For instance, the state 1-state 2 transition enables to change distribution of the excitation energy between photosystems and maximize the efficiency of photosynthesis [1]. Recently, energy dissipation mechanism triggered by intense blue light (around 470 nm) has also been reported as the blue light-induced quenching [2]. These light adaptations depend on the quality and the quantity of light. In this study, we examined the excitation energy transfer processes in cells exposed to high light exhibiting different light qualities, by means of time-resolved fluorescence spectroscopy.

Intact cells of PCC 6803 were illuminated with blue (460 nm, B), green (515 nm, G), orange (640 nm, R1), red (660 nm, R2), and white (W) LEDs (light-emitting diodes) with a light intensity of 500  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  for 5 minutes. Dark-adapted cells were prepared as a control sample. Steady-state absorption spectra, steady-state fluorescence spectra, and time-resolved fluorescence spectra (TRFS) in the picosecond to nanosecond time range were measured at 77 K.

There was no detectable change in the steady-state absorption spectra. This indicates that the pigment composition was conserved among all samples. In the steady-state fluorescence spectra, relative intensities of APC to PC in R1- and R2-illuminated cells were almost the same as the dark-adapted cells (R-group), while, in B-, G-, and W-illuminated cells (B-group), those were decreased to 70-80% of that in the dark-adapted cells. In the TRFS, the considerable reductions in relative intensities of APC were found only in the B-group, which was noticeable especially in the time region of 200 ps-1.2 ns after excitation. This change might assign to the blue light-induced quenching, since B-, G-, and W-LEDs commonly have emission intensities around 470 nm. We will discuss light-adaptation processes observed as changes in TRFS, using fluorescence decay-associated spectra.

[1] W. Patrick Williams, John F. Allen, *Photosynthesis Research*, 13 (1987) 19-45; [2] Marina G. Rakhimberdieva, Igor N. Stadnichuka, Irina V. Elanskayab, Navassard V. Karapetyana, *FEBS Letters*, 574 (2004) 85-88.

### **P132: Investigation of the symbiotic interaction between *Chlamydomonas intermedia* and *Brevundimonas* sp. consortium**

**Gergely E. Lakatos<sup>1,2</sup>, Kata Keresztúri<sup>2</sup>, Balázs Horváth<sup>2</sup>, Gergely Maróti<sup>1,2</sup>**

<sup>1</sup>Bay Zoltán Nonprofit Research Ltd., Szeged, Hungary; <sup>2</sup>Institute of Biochemistry, Biological Research Centre, Szeged, Hungary

The presumed symbiotic interaction between *Chlamydomonas intermedia* and various bacterial strains was investigated in details. The green algae *Chlamydomonas intermedia* MACC-549 has been cultivated in Kuhl liquid medium for decades. During cultivation different types of consortia including *Chlamydomonas intermedia* MACC-549 were established. By plating serial dilutions different non-algal microorganisms were identified as essential members of these microbial communities.

Characterization based on 16S rDNA sequence analysis and full genome sequencing showed that all of the bacterial partners belong to *Brevundimonas* genus. They were named Brev. Y., Brev. LY. and Brev. O. The use of antibiotics in various combinations made possible the selective growth of the algae itself and all combinations between the algae and the *Brevundimonas* strains.

We have investigated the effects of the presence of various combinations of the identified bacteria on the physiological and metabolic properties of the *Chlamydomonas intermedia* MACC-549 algae. Metabolic alterations were monitored by HPLC by measuring acetate, formate, lactate and ethanol in the microbial communities in line with continuous pH measurements. Using air-tight serum bottles the headspace of the cultures was monitored by gas chromatography, characteristic fluctuations were detected in the level of nitrogen, oxygen and hydrogen for each combination.

Whole transcriptome analyses of the single and symbiotic cultures were performed in order to detect the differential expression of several bacterial and algae genes, therefore to understand the intracellular metabolic processes responsible for the observed specific phenotypes of mixed cultures.



### P133: Studies on the reduction processes from chlorophyll *a* esterified with geranylgeraniol to that with $\Delta$ 2,6-phytyadienol in the green sulfur bacterium *Chlorobaculum tepidum*

Hirozo Oh-oka<sup>1</sup>, Chihiro Azai<sup>2</sup>, Jiro Harada<sup>3</sup>, Syohei Miyago<sup>1</sup>, Tadashi Mizoguchi<sup>4</sup>, and Hitoshi Tamiaki<sup>4</sup>

<sup>1</sup>Graduate School of Science, Osaka University, Japan; <sup>2</sup>Graduate School of Science, Nagoya University, Japan; <sup>3</sup>Kurume University School of Medicine, Japan; <sup>4</sup>Graduate School of Life Sciences, Ritsumeikan University, Japan

(Bacterio)chlorophylls [(B)Chls] not only constitute various light-harvesting apparatuses which enable photosynthetic organisms to capture light energy at a high efficiency, but also serve as cofactors involved in initial charge separations in photosynthetic reaction centers (RCs). In green sulfur bacterial RC complex, Chls  $a_{pd}$  are supposed to function as a primary electron acceptor as well as accessory pigments [1]. Chl  $a_{pd}$  has the same chlorin  $\pi$ -system as Chl  $a_p$  in higher plants and cyanobacteria except for a long hydrocarbon chain at the C-17 position; the former is esterified with  $\Delta$ 2,6-phytyadienol and the latter is with phytol [2]. Geranylgeraniol is a precursor of phytol, and three of its four double bonds are stepwisely reduced by geranylgeranyl reductase (GGR) [3]. Previously, we demonstrated that the *CT2256* gene in *Chlorobaculum tepidum* encoded GGR carrying out hydrogenation of geranylgeranyl (GG) group by constructing its disruption mutant using an insertional inactivation method and was found to be responsible for the production of both  $\Delta$ 2,6-phytyadienyl and phytyl groups [3]. However, the reaction process to produce  $\Delta$ 2,6-phytyadienyl ester is still unknown.

In the present study, we incorporated cyanobacterial and purple bacterial GGR genes, that is, *chlP* and *bchP* genes, respectively, into the *CT2256*-deletion mutant by replacing the disrupted site of the relevant gene on the genome. In the *chlP*-incorporated strain, BChl  $a_p$  as well as intermediates synthesized during the reduction process of BChl  $a_{GG}$  were accumulated in addition to Chl  $a_p$ . From the HPLC analysis, the order of the reductions of the GG group bound to BChl  $a$  was found to be the same as that in purple bacteria. On the other hand, in the *bchP*-incorporated strain, Chl  $a_p$  as well as intermediates were accumulated in addition to BChl  $a_p$ . However, the order of the reductions of the GG group bound to Chl  $a$  was different from that bound to BChl  $a$ , and Chl  $a_{pd}$  was detected unexpectedly. These data has suggested that the *CT2256* might discriminate the difference of ring structures between chlorin and bacteriochlorin and produce BChl  $a_p$  and Chl  $a_{pd}$  from BChl  $a_{GG}$  and Chl  $a_{GG}$ , respectively. In order to clarify specific properties of the *CT2256*, the complementary experiments are now in progress using the *chlP*-deletion mutant of *Synechocystis* sp. PCC6803.

[1] M. Iwaki, S. Itoh, S. Kamei, H. Matsubara and H. Oh-oka (1999) *Plant Cell Physiol.* 40: 1021-1028; [2] M. Kobayashi, H. Oh-oka, S. Akutsu, M. Akiyama, K. Tominaga, H. Kise, F. Nishida, T. Watanabe, J. Amesz, M. Koizumi, N. Ishida and H. Kano (2000) *Photosynth. Res.* 63: 269-280; [3] T. Mizoguchi, J. Harada and H. Tamiaki (2006) *FEBS Lett.* 580: 6644-6648; [4] J. Harada, S. Miyago, T. Mizoguchi, C. Azai, K. Inoue, H. Tamiaki and H. Oh-oka (2008) *Photochem. Photobiol. Sci.* 7: 1179-1187.

### P134: Detection of reactive oxygen species in cultured symbiotic dinoflagellate algae (genus *Symbiodinium*)

Ateeq ur Rehman<sup>1</sup>, Zsuzsanna Deák<sup>1</sup>, Anthony Larkum<sup>2</sup> and Imre Vass<sup>1</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary; <sup>2</sup>Plant Functional Biology and Climate Change CLUSTER, Department of Environmental Sciences, University of Technology, Sydney, PO Box 123, Broadway, NSW 2007, Australia

Scleractinian corals contain symbiotic dinoflagellates from the genus *Symbiodinium*. Photosynthetic products of these symbionts provide an essential carbon energy source for the coral host, and the coral host supplies essential nitrogen, growth factors, respiratory by-products, as well as protection. Light stress in combination with elevated temperatures can disrupt this symbiosis which often results in the expulsion of the symbiotic algae from the host tissue leading to coral bleaching. The photosynthetic apparatus of the zooxanthellae has been thought to be an important site of breakdown in the symbiosis, and previous studies have indicated that reactive oxygen species (ROS), such as superoxide [1] might be involved in the process. The most damaging ROS in photosynthetic systems is singlet oxygen ( $^1O_2$ ), which is produced via interaction of Chl triplets, that are formed in the Photosystem II complex, and in the light harvesting antenna [2]. So far  $^1O_2$  has not been detected in *Symbiodinium*, mainly do to the lack of reliable  $^1O_2$  sensor that can penetrate inside the cells.

Here we report the first observations about light induced  $^1O_2$  production in cultured *Symbiodinium* cells, by using histidine-mediated oxygen uptake measurements. First, we demonstrated that in the presence of the known  $^1O_2$  sensitizer Rose Bengal His-mediated oxygen uptake occurs in cell free culture medium, which is suppressed by the  $^1O_2$  quencher  $NaN_3$ , and enhanced by  $D_2O$ , that increases the lifetime of  $^1O_2$ . When the His assay was applied to a suspension of cultured *Symbiodinium* cells we also observed light-induced, His-mediated oxygen uptake, which was significantly increased in the presence of  $D_2O$ . These data show that ROS, with a significant contribution by  $^1O_2$ , is formed in illuminated *Symbiodinium* cells. ROS production in photosynthetic organisms depends on the efficiency of the electron sinks, especially of  $CO_2$  fixation. When the Calvin-Bensson cycle was inhibited by the addition of glycolaldehyde, His-mediated oxygen uptake was increased, which shows enhanced ROS production in the presence of reduced electron transport chain. Similar effect was observed after exposure of cells to elevated temperature in combination with increased light intensity, which together mimic conditions that can trigger coral bleaching.

Our data demonstrate light induced production of ROS ( $^1O_2$ ), in cultured *Symbiodinium* cells. We can also conclude that ROS ( $^1O_2$ ) production, which is enhanced by inhibition of the Calvin-Bensson cycle, may be an important trigger for expelling zooxanthellae from coral tissue in the process of coral bleaching.

[1] D. Tchernov, M. Y. Gorbunov, C. de Vargas, S. N. Yadav, A. J. Milligan, M. Häggblom, and P. G. Falkowski (2004) *Proc. Natl. Acad. Sci. USA* 101 13531-13535; [2] A. Krieger-Liszskay (2005) *J. Exp. Bot.* 56 337-346.

## P135: The YCF39-like product of the *slrO399* gene is a component of the *Synechocystis* Photosystem II reaction center complex lacking antennae CP47 and CP43

Jana Knoppová<sup>1,2</sup>, Peter J. Nixon<sup>3</sup> and Josef Komenda<sup>1,2</sup>

<sup>1</sup>Laboratory of Photosynthesis, Institute of Microbiology, Třeboň, Czech Republic; <sup>2</sup>Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic; <sup>3</sup>Division of Molecular Biosciences, Imperial College London, United Kingdom

Assembly of the Photosystem II (PSII) complex in the model cyanobacterium *Synechocystis* sp. PCC6803 proceeds via a number of assembly subcomplexes. When the PSII antenna CP47 cannot be synthesized, low level of two reaction centre (RC)-like subcomplexes containing D1, D2, PsbE, PsbF, PsbI subunits and an assembly factor YCF48 accumulates in the membranes. These subcomplexes differ in size and mass spectrometric analysis of these subcomplexes separated by two-dimensional clear native/SDS-PAGE identified the *slrO399* gene product as an additional component of the "larger" RC complex termed RC\*. This 36 kDa protein is very similar to the hypothetical protein YCF39 encoded in the chloroplast genome of some algae and in the nuclear genome of plants. This protein has been previously considered as quinone chaperone due to its effect on function of the PSII primary quinone acceptor Q<sub>A</sub>.

We unequivocally confirmed the association of SlrO399 with RC\* by copurification of this subcomplex using FLAG-tagged SlrO399 expressed in a strain lacking both CP47 and the native SlrO399. The presence of the PSII RC protein components in the eluate was proved both by mass spectrometry and by 2D PAGE in combination with Western blotting. Moreover, two members of Scp family, ScpB and especially ScpE were also found in the preparation. The proteins of this family are thought to transiently bind carotenoids and chlorophyll, and to regulate their biosynthesis. Analysis of the CP47-lacking strain by 2D PAGE revealed that SlrO399 and ScpE also formed a small complex. The absence of ScpE in this strain resulted in a substantial decrease of the amount of RC\* subcomplex and relocation of the majority of SlrO399 to unassembled protein fraction. SlrO399 was also present in an unidentified complex with size between those of SlrO399-ScpE and RC\* complexes. On the other hand, SlrO399 was absent in larger PSII complexes including the CP43-lacking subcomplex RC47.

Phenotype of the  $\Delta$ SlrO399 mutant did not differ from that of WT strain under normal growth conditions (30°C, 40  $\mu$ E m<sup>-2</sup>s<sup>-1</sup>). However, the radioactive protein labelling revealed the absence of newly synthesized RC\* subcomplex in the mutant. After transferring the cells to photoinhibitory irradiance (700  $\mu$ E m<sup>-2</sup>s<sup>-1</sup>), the oxygen evolving activity of PSII dropped down more than twice in the mutant in comparison to WT, documenting the importance of *de novo* assembly of PSII for the efficient coping with the high light stress. The results showed that SlrO399 represents a transiently bound protein factor associated with early stages of PSII biogenesis. The association of SlrO399 with the ScpE, a protein putatively binding tetrapyrrol pigments and regulating their biosynthesis, implies its relation to the insertion of these pigments rather than quinone into PSII RC.

## P136: Physiological tolerance of cyanobacteria for hydrocarbon fuel production

Jari Kämäräinen, Fernando Guerrero, M. Kalim Akhtar, Eva-Mari Aro and Patrik R. Jones

University of Turku, Molecular Plant Biology, Tykistökatu 6A, 20520 Turku, Finland

The search for sustainable renewable fuel production technologies is essential on both a global and local level as the consumption of fuel continues to rise in tandem with the growing population (Tollefson, 2011). Since mature and large-scale technologies for production of renewable electricity already exist, the grand challenge is to produce fuels (~85% of total energy use) in a sustainable manner and at a scale that would allow fossil fuels to eventually be phased out. The benefits with aquatic systems, compared to terrestrial plants, are many-fold, including reduced restrictions on water quality, no requirement for arable land, faster biomass-accumulation and lower metabolic maintenance costs (Searchinger et al., 2008). The first generation of aquatic-based systems is likely to be based on native capabilities for production of relatively inert native precursors such as polysaccharides (Mussgnug et al., 2010) and triglyceride. Such precursors will require further abiological processing or fermentative conversion into fuel, possibly in conjunction with thermo-chemical strategies.

Cyanobacteria are also capable of the direct conversion of sunlight, carbon dioxide and water into hydrocarbon fuel, as was illustrated for ethylene (Takahama et al., 2003), isoprene (Lindberg et al., 2009), ethanol (Deng and Coleman, 1999) and butanol (Lan and Liao, 2011). The relative tolerance of cyanobacteria to different fuels is still an open question, however. The effect of exogenous addition of a selection of current fuel targets and their precursors on the growth of *Synechocystis* sp. PCC 6803 and *Synechococcus elongatus* PCC 7942 was therefore evaluated. Externally added alcohols were by far the most toxic, followed by aldehydes and acids, whilst alkanes were the least toxic. The toxicity became progressively greater with increasing chain-length for alcohols whilst the intermediate C6 alkane was more toxic than both C3 and C11 alkane. *Synechocystis* sp. PCC 6803 was more tolerant to some of the tested chemicals than *Synechococcus elongatus* PCC 7942, particularly for ethanol and undecane. In summary, it was concluded that alkanes would constitute the best choice metabolic end-product for fuel production using cyanobacteria if high-yielding strains can be developed.

Deng, M., & Coleman, J. (1999) Ethanol synthesis by genetic engineering in cyanobacteria. 65, 523-528; Lan, E. I., & Liao, J. C. (2011) Metabolic engineering of cyanobacteria for 1-butanol production from carbon dioxide. 13, 353-363; Lindberg, P., Park, S., & Melis, A. (2009) Engineering a platform for photosynthetic isoprene production in cyanobacteria, using *synechocystis* as the model organism; Mussgnug, J. H., Klassen, V., Schluter, A., & Kruse, O. (2010) Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. 150, 51-56; Searchinger, T., Heimlich, R., Houghton, R. A., Dong, F., Elobeid, A., Fabiosa, J., Tokgoz, S., Hayes, D., & Yu, T. H. (2008) Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. 319, 1238-1240; Takahama, K., Matsuoka, M., Nagahama, K., & Ogawa, T. (2003) Construction and analysis of a recombinant cyanobacterium expressing a chromosomally inserted gene for an ethylene-forming enzyme at the *psbAI* locus. 95, 302-305; Tollefson, J. (2011) Seven billion and counting. Nature. 2011 Oct 19;478(7369):300.

## P137: Maturation of the pilin protein PilA1 is essential for autotrophic growth of *Synechocystis* PCC 6803

Marketa Foldynova<sup>1,2</sup>, Josef Komenda<sup>1</sup>, Martin Tichy<sup>1,2</sup> and Roman Sobotka<sup>1,2</sup>

<sup>1</sup>Institute of Microbiology, Trebon, Czech Republic; <sup>2</sup>Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic.

Pilins (PilA proteins) are subunits of Type IV pili – fibres that occur on surface of many bacteria including cyanobacteria. Essential for bacterial cell adhesion, motility, and DNA uptake, pilins in cyanobacteria have also been proposed to participate in biogenesis of chlorophyll-binding proteins [1]. Genome of the cyanobacterium *Synechocystis* PCC 6803 contains multiple genes encoding for various PilA homologues. Although role of most of them remains unknown, their proper function depends on a single enzyme - PilD peptidase, which matures PilA proteins by removal of signal sequence.

As a tool to study function of PilA proteins in cyanobacteria, we used *Synechocystis*  $\Delta pilD$  strain that lacks all mature PilA proteins. Using specific antibodies, we proved that the strain accumulated unprocessed PilA1 (prePilA1) protein and its degradation product. Interestingly, the  $\Delta pilD$  strain lost capability of autotrophic growth and grew only in the presence of glucose. After removal of glucose,  $\Delta pilD$  cells quickly lost precursors for chlorophyll biosynthesis (in 24 hours) followed by a rapid decrease in cellular content of phycobiliproteins, Photosystem II and later also Photosystem I. The  $\Delta pilD$  strain formed very frequently autotrophic pseudorevertants and thus to identify a  $\Delta pilD$  suppressor mutation, the genome of an autotrophic pseudorevertant was completely sequenced. We found that the sequenced strain harbours a point mutation causing amino-acid substitution in SigF - a sigma factor controlling transcription of PilA1 and PilA2 proteins [2]. Indeed, this mutation led to strong decrease in prePilA1 protein level when compared to the original  $\Delta pilD$  mutant. Analysing more pseudorevertants we found that their capacity to grow autotrophically is negatively correlated with the accumulation of prePilA1. In order to confirm that the high level of prePilA1 is responsible for the loss of autotrophy in the  $\Delta pilD$  strain, we prepared  $\Delta pilD/\Delta pilA1$  strain in which the autotrophy was fully restored.

Importance of the PilA1 protein for cyanobacterial photosynthesis was further supported by phenotype of the  $\Delta pilA1$  strain. Under low light conditions, the  $\Delta pilA1$  was akin the  $\Delta pilD$  strain to some extent due to the reduced level of photosynthetic complexes while no such phenotype was observed in other *Synechocystis*  $\Delta pilA$  mutants ( $\Delta pilA2$ ,  $\Delta pilA4$ ,  $\Delta pilA5-8$ ,  $\Delta pilA9-11$ ) or in mutants lacking factors important for pili biogenesis ( $\Delta pilB1$ ,  $\Delta pilC$ ,  $\Delta pilQ$ ,  $\Delta pilT$ ).

[1] He Q, Vermaas WF (1999) Plant Mol Biol 39:1175-1188, [2] Bhaya D, et al. (1999) PNAS 96:3188-3193.

.....

**P138: The respiratory terminal oxidases of the cyanobacterium *Anabaena* sp. strain PCC7120****Markus Mikulic<sup>1</sup>, Ronald Stebegg<sup>1</sup>, Ana Valladares<sup>2</sup>, Enrique Flores<sup>2</sup>, and Georg Schmetterer<sup>1</sup>**<sup>1</sup>Institute of Physical Chemistry, University of Vienna, Vienna, Austria; <sup>2</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Sevilla, Spain

The respiratory terminal oxidases (RTOs) of cyanobacteria are the key enzymes of respiration, because they are the only components of the respiratory chain(s) not also directly involved in photosynthetic electron transport. The total genomic sequence of *Anabaena* (also known as *Nostoc*) sp. strain PCC7120 has revealed that this organism contains five different RTOs: 1) a mitochondrial-type cytochrome *c* oxidase (Cox), 2) two ARTOs, enzymes homologous to Cox but with characteristic sequence differences [1], 3) a putative quinol oxidase (Qox) homologous to cytochrome *bd* from *Escherichia coli*, and 4) a putative plastidic-type quinol oxidase (Ptox). Our goal is to define a specific function for each of the RTOs. We now have available homozygous knock-out mutants for each of the 5 RTOs. This implies that none of the RTOs is essential for phototrophic growth. The two ARTOs are involved in heterocyst development and probably act as scavengers for O<sub>2</sub> in heterocysts [2]. The Cox is essential for dark growth of this strain (manuscript submitted). The function of the Qox and the Ptox remains unclear. The apparent respiratory rate of the Qox mutant cells is significantly higher than in wild type cells when calculated as  $\mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot [\text{mg chlorophyll}]^{-1}$ . This is caused mainly by the considerably lower content of chlorophyll per cell in the Qox mutant and only partly by a higher O<sub>2</sub> consumption rate. In contrast, mutants lacking the Cox have an increased content of chlorophyll per cell. Since the Cox mutant has almost the same respiratory rate (in  $\mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot [\text{mg chlorophyll}]^{-1}$ ) as the wild type, the O<sub>2</sub> consumption of the Cox mutant is actually higher than that of the wild type. Apparently another RTO overcompensates the loss of the Cox. When calculated as  $\mu\text{mol O}_2 \cdot \text{h}^{-1} \cdot [\text{OD}_{730}]^{-1}$  the respiratory rates of all RTO mutants investigated is higher than in wild type cells so that this overcompensation may be a general response to loss of one of the RTOs. The chloroplast Ptox is resistant against inhibition by KCN. This is apparently not the case for the Ptox of *Anabaena*. The Qox mutant has an unusual phenotype against KCN. While in all strains containing Qox the inhibition by KCN is only manifest after 8-10 minutes, strains lacking the Qox are immediately (within 1 minute) inhibited by KCN (0.67 mM). In *Synechocystis* sp. PCC6803 HQNO inhibits specifically and completely the Qox. An *Anabaena* mutant lacking both Cox and Ptox grown on nitrate is expected to have only Qox as its RTO, however, HQNO inhibits respiration by only about 35%, not significantly more than in the wild type.

[1] Schmetterer G., Pils D.: Cyanobacterial respiration. In: Zannoni D. (ed) Respiration in Archaea and Bacteria. Vol 2: Diversity of prokaryotic respiratory systems. pp. 261-278 (2004); [2] Valladares A., Herrero A., Pils D., Schmetterer G., Flores E.: Cytochrome *c* oxidase genes required for nitrogenase activity and diazotrophic growth in *Anabaena* sp. PCC7120. Mol. Microbiol. 47: 1239-1249 (2003).

**P139: Whole-genome resequencing of *Synechocystis* sp. PCC 6803 strains****Martin Tichy<sup>1,2</sup>**<sup>1</sup>Department of Phototrophic Microorganisms, Institute of Microbiology, Academy of Sciences, Trebon, Czech Republic, tichym@alga.cz; <sup>2</sup>Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

*Synechocystis* sp. PCC 6803 belongs to the most popular cyanobacterial strains, serving as a model cyanobacterium in many laboratories around the world for 40 years. In 1996, its genome sequence was determined as the first genome of photosynthetic organism and among the first sequenced prokaryotes.

Over the years of isolated cultivation in individual laboratories, phenotypically different variants of the original *Synechocystis* 6803 strain were selected and several mutations responsible for these differences were described. Current high-throughput next-generation sequencing technologies allow cost-efficient, reference-based resequencing of the whole *Synechocystis* 6803 genome to reveal complete genetic background of individual strain variants.

We present results of resequencing of the 12 strain variants of two phenotypically distinct laboratory lines of *Synechocystis* 6803 from labs of Wim Vermaas and Peter Nixon. These variants include original strains, various mutants and second-site revertants with improved phenotype. From the analysis of this set of data, several conclusions can be drawn:

- Despite relatively long separation of both lines only a few differences were determined.
- Substitution is the most common mutation. Interestingly, although *Synechocystis* 6803 has a multi-copy genome, some sense mutations got segregated.
- Large genome rearrangement by tandem duplication was observed in two variants. Transposons probably play a role in this process.
- In cases where it is particularly difficult to get a fully segregated mutant, like deletion of *psbA* or deletion of Photosystem I, secondary complementing mutation(s) are necessary before full segregation is reached.
- Different mutations in the *gene slr1609* in different lines suggest that mutation in this gene is advantageous for laboratory cultivation of *Synechocystis* 6803.

## **P140: Localization and possible function of the Psb28-2 protein in the biogenesis of the Photosystem II complex in the cyanobacterium *Synechocystis* sp. PCC 6803**

**Martina Beckova<sup>1,2</sup>, Roman Sobotka<sup>1</sup>, Martin Tichy<sup>1</sup>, Peter J. Nixon<sup>3</sup> and Josef Komenda<sup>1,2</sup>**

<sup>1</sup>Institute of Microbiology, Trebon, Czech Republic; <sup>2</sup>Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic; <sup>3</sup>Division of Molecular Biosciences, Imperial College London, United Kingdom

Biogenesis of the cyanobacterial Photosystem II complex (PSII) is a step-wise process during which several distinct assembly intermediate subcomplexes can be detected. One of them is a core complex lacking the CP43 antenna termed RC47, which consists of the D1, D2, CP47 and a number of low molecular mass subunits. We have previously shown that RC47 also binds a protein factor Psb28 (encoded by the *sll1398* gene), which affects by unknown mechanism synthesis of Chl binding proteins, especially CP47. Genome of *Synechocystis* and some other cyanobacterial strains contains a gene similar to *psb28* (*sll1398*), designated *psb28-2* (*sll1739*). The aim of this study was to establish the relationship between Psb28 and Psb28-2 as concerns their localization and function in the biogenesis of photosynthetic membrane complexes.

For Psb28-2 localization, we initially constructed a mutant expressing the N-terminally FLAG-tagged Psb28-2 protein under *petJ* promoter. Using two-dimensional clear-native/SDS-PAGE in combination with Flag detection, the tagged Psb28-2 protein was found associated with RC47 and unlike Psb28 also with the complete monomeric PSII core complex (RCC1). This localization was confirmed in a strain expressing C-terminally Flag-tagged Psb28-2 under native promoter and finally also in the *psb28* deletion mutant using antibody specific for native Psb28-2. These data indicated a similar binding properties of Psb28 and Psb28-2 in respect to PSII complexes with the former having higher affinity for RC47 while the latter for RCC1. The wild type strain (WT) contained only small amount of RC47 and consequently only small fraction of Psb28 was associated with this complex while Psb28-2 was hardly detectable outside of any PSII complex. When the cells were exposed to high irradiance in the absence and presence of protein synthesis inhibitor, the increase in the level of RC47 was accompanied by an increased association of Psb28 with RC47 and Psb28-2 with RCC1, but only in the presence of the inhibitor. Neither Psb28, nor Psb28-2 was detected in PSII complexes in the strains lacking the small CP47-associated protein PsbH indicating their similar binding site on CP47 near PsbH like ScpC/D, small chlorophyll a/b binding-like proteins induced under high irradiance. Interestingly, Psb28 and Psb28-2 could bind to PSII complexes neither in the control, nor in high light- and tetracycline-treated cells of PSI-less strains, unless genes for Scp proteins were inactivated. The results indicate competition between Psb28, Psb28-2 and Scp proteins for the similar binding site on CP47 within PSII. This competition can indirectly modify chlorophyll biosynthesis via affecting the level of those forms of Scps that regulate synthesis of chlorophyll intermediates.

## **P141: Expression of the ferredoxin C-terminal CAB domain in the cyanobacterium *Synechocystis* sp. PCC 6803 to determine its role in tetrapyrrole biosynthesis**

**Petra Skotnicova<sup>1,2</sup>, Roman Sobotka<sup>1,2</sup>, Josef Komenda<sup>1,2</sup>, and Martin Tichy<sup>1,2</sup>**

<sup>1</sup>Department of Phototrophic Microorganisms, Institute of Microbiology, Academy of Sciences, Trebon, Czech Republic skotnicova@alga.cz; <sup>2</sup>Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic

Ferredoxin, the final enzyme of heme biosynthesis catalyzes insertion of ferrous ion into protoporphyrin IX. In plant chloroplasts and in cyanobacteria, ferredoxin lies at the branch-point of heme/chlorophyll biosynthesis. Characteristic feature of the cyanobacterial and one of the chloroplast ferredoxins is their C-terminal hydrophobic domain with a high degree of similarity to the first and third helices of the plant LHCII light harvesting complex including a Chl-binding motif (CAB domain). We have shown recently that the CAB domain serves mainly a regulatory function, possibly in balancing Chl biosynthesis with the synthesis of cognate apoproteins [1].

Using strains expressing the CAB domain as a separate protein we will show that ferredoxin interacts with an early Photosystem II complex and that the CAB domain itself can complement phenotype of the ferredoxin CAB truncation mutant. We will also present latest data on anticipated pigment binding to the ferredoxin CAB domain.

[1] Sobotka, et al. (2011) Plant Physiol., 155, 1735–1747.



### P143: Genetic Engineering in *Synechocystis* sp. PCC 6803 for Solar Hydrogen Production

Karen Wawrousek, Scott Noble, Jianping Yu, and Pin-Ching Maness

Biosciences Center, National Renewable Energy Laboratory, Golden, Colorado 80401 USA

The cyanobacterium *Synechocystis* sp. PCC 6803 produces H<sub>2</sub> during photosynthesis by employing a Hox bidirectional [NiFe]-hydrogenase. However, the H<sub>2</sub>-production reaction is short-lived in O<sub>2</sub>, the latter an inherent byproduct of oxygenic photosynthesis<sup>1</sup>. To surmount this challenge, we aim to construct a *Synechocystis* host expressing a foreign, O<sub>2</sub>-tolerant hydrogenase. Prior research in our laboratory has uncovered an O<sub>2</sub>-tolerant [NiFe]-hydrogenase from the purple non-sulfur photosynthetic bacterium *Rubrivivax gelatinosus* CBS (hereafter "CBS")<sup>2</sup>. We have cloned the genes *cooMKLXUH*, which encode the hexameric hydrogenase complex in CBS. Amino acid alignment suggests this hydrogenase belongs to the Group 4 energy-conserving hydrogenase with a physiological function in H<sub>2</sub> production from CO according to the equation CO + H<sub>2</sub>O → H<sub>2</sub> + CO<sub>2</sub><sup>3</sup>. Additionally, six genes, *hypABCDEFGF*, were identified with putative roles in the assembly and maturation of the hydrogenase catalytic subunit. Based on protein immunoblot and qRT-PCR, expression of both the hydrogenase structural subunits and the *hyp* maturation genes are induced by CO, but not by its product H<sub>2</sub>, in CBS. This leads to the discovery of a CO-sensing transcription regulator RcoM clustered amongst the *hyp* genes. Upon deletion of *rcoM*, neither the hydrogenase nor the maturation proteins are produced in CO, with a concomitant loss of CO-supported cell growth and H<sub>2</sub> production in CBS. This is the first evidence that CO regulates the expression of a set of hydrogenase maturation machinery, which strongly suggests their role in assembling the CO-linked hydrogenase. Four *Rubrivivax* hydrogenase genes, *cooLXUH*, and the six maturation genes, *hypABCDEFGF*, were transformed into a *Synechocystis* host lacking the native Hox hydrogenase. We determined that the heterologously expressed *CooLXUH* formed a soluble complex in the *Synechocystis* host, albeit with no activity. Work is ongoing to further elucidate the role of RcoM in CBS and to optimize CBS hydrogenase expression in *Synechocystis*.

(1) Cournac, L., Guedeney, G., Peltier, G., and Vignais, P.M. 2004. Sustained photoevolution of molecular hydrogen in a mutant of *Synechocystis* sp. PCC 6803 deficient in the type I NADPH-dehydrogenase complex. *J. Bacteriol.* 186, 1737-1736; (2) Maness, P.C., Smolinski, S., Dillon, D.C., Heben, M.J., and Weaver, P.F. 2002. Characterization of the oxygen tolerance of a hydrogenase linked to a carbon monoxide oxidation pathway in *Rubrivivax gelatinosus*. *Appl. Environ. Microbiol.* 68: 2633-2636; (3) Vanzin, G., Yu, J., Smolinski, S., Tek, V., Pennington, G., and Maness, P.C. 2010. Characterization of genes responsible for the CO-linked hydrogen production pathway in *Rubrivivax gelatinosus*. *Appl. Environ. Microbiol.* 76: 3715-3722.

### P144: The CyanoNetwork: Towards an integrative computational description of cyanobacterial growth

Ralf Steuer<sup>1,2</sup>

<sup>1</sup>CzechGlobe - Global Change Research Centre AS CR, v.v.i., Czech Republic; <sup>2</sup>Institute for Theoretical Biology, Humboldt-University of Berlin, Berlin, Germany

Cyanobacteria are phototrophic microorganisms of global importance and have recently attracted increasing attention due to their capability to convert sunlight and atmospheric CO<sub>2</sub> into organic compounds, including carbon-based biofuels. Our ability to fully harness the biotechnological potential of cyanobacteria would greatly benefit from an increasing understanding of intracellular processes that determine cyanobacterial growth. Such understanding is increasingly facilitated by the construction of computational models of the respective organisms. From a modelling perspective, cyanobacteria are highly attractive organisms whose computational description allows to integrate a large number of challenging questions. In particular, a computational description of phototrophic growth must span a hierarchy of processes, ranging from the biophysics of photosynthesis, the biochemistry of carbon fixation, molecular mechanisms of cellular growth, marine ecology, to global cycles of oxygen and carbon. The integration of such diverse processes is clearly beyond the capabilities of a single research group and requires a multi-disciplinary effort. As a first step towards an integrative computational description of cyanobacterial growth, we have therefore established the CyanoNetwork – an association of scientist from diverse backgrounds dedicated to contribute to the construction of a cyanobacterium *in-silico*. The contribution will provide an overview on these activities and will describe recent efforts to integrate these diverse layers of phototrophic growth into a coherent whole.

[1] R. Steuer, H. Knoop and R. Machne (2012) Modelling cyanobacteria: from metabolism to integrative models of phototrophic growth. *Journal of Experimental Botany* 63(6):2259-74, doi:10.1093/jxb/ers018; [2] C. Beck, H. Knoop, IM Axmann, R. Steuer (2012) The diversity of cyanobacterial metabolism: genome analysis of multiple phototrophic microorganisms. *BMC Genomics*. 2012 Feb 2;13(1):56; [3] Safránek D, Cervený J, Klement M, Pospíšilová J, Brim L, Lazár D, Nedbal L. E-photosynthesis: web-based platform for modeling of complex photosynthetic processes. *Biosystems*. 2011 Feb;103(2):115-24.

### P145: Light-dependent and asynchronous replication of cyanobacterial multi-copy chromosomes

Satoru Watanabe<sup>1</sup>, Ryudo Ohbayashi<sup>1</sup>, Yuh Shiwa<sup>2</sup>, Asuka Noda<sup>1</sup>, Yu Kanesaki<sup>2</sup>, Taku Chibazakura<sup>1</sup>, and Hirofumi Yoshikawa<sup>1,2</sup>

<sup>1</sup>Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan; <sup>2</sup>Genome Research Center, NODAI Research Institute, Tokyo University of Agriculture, Tokyo, Japan

While bacteria such as *Escherichia coli* and *Bacillus subtilis* harbor a single circular chromosome, some freshwater cyanobacteria have multiple chromosomes per cell. The detailed mechanism(s) of cyanobacterial replication remains unclear. We show here the replication origin (*ori*), form, and synchrony of the multi-copy genome in freshwater cyanobacteria *Synechococcus elongatus* PCC 7942. Mapping analysis of nascent DNA fragments using a next-generation sequencer indicated that replication starts bidirectionally from a single *ori*, which locates in the upstream region of the *dnaN* gene. In addition, the replication is initiated asynchronously not only among cell populations but also among the multi-copy chromosomes. Our findings suggest that replication initiation is regulated less stringently in *S. 7942* than in *E. coli* and *B. subtilis*.

[1] Watanabe S., Ohbayashi R., Shiwa Y., Noda A., Kanesaki Y., Chibazakura T., and Yoshikawa H., Light-dependent and asynchronous replication of cyanobacterial multi-copy chromosomes. *Mol. Microbiol.* (2012) 83: 856-865.



## P146: Energy transfer in a symbiotic cyanobacterium, *Prochloron*, probed by *in hospite* spectroscopic measurements

Fumiya Hamada<sup>1</sup>, Makio Yokono<sup>2,3</sup>, Euichi Hirose<sup>4</sup>, Akio Murakami<sup>1,5</sup>, and Seiji Akimoto<sup>1,2,3</sup>

<sup>1</sup>Graduate School of Science, Kobe University, Kobe, Japan; <sup>2</sup>Molecular Photoscience Research Center, Kobe University, Kobe, Japan; <sup>3</sup>JST CREST, Kobe, Japan; <sup>4</sup>Faculty of Science, University of the Ryukyus, Nishihara, Okinawa, Japan; <sup>5</sup>Kobe University Research Center for Inland Seas, Awaji, Japan

Most of cyanobacteria have chlorophyll *a* and phycobilins as major photosynthetic pigments, but some species of cyanobacteria have chlorophylls other than chlorophyll *a* and lack phycobilisomes. *Prochloron* sp. possesses Chl *b* in addition to Chl *a*, but does not have phycobilisomes. Its photosystem antennae comprise three types of Pcb (Prochlorophyte chlorophyll-binding protein), PcbA, PcbB, PcbC, whereas Chl *a/b*-containing green algae and higher plants possess eukaryotic-type antennae, LHC (light-harvesting chlorophyll protein complex) I and LHC II. *Prochloron* is anomalous in that it lives as an obligate symbiont in colonial ascidians inhabiting tropical/subtropical coral reefs; free-living *Prochloron* has not been reported to date [1]. Moreover, *Prochloron* cannot be cultured *in vitro* reliably at present. We investigated differences in light-harvesting strategies of *Prochloron* in the four ascidian species, *Diplosoma* sp., *Trididemnum cyclops*, *Trididemnum miniatum*, and *Lissoclinum timorense*, by means of *in hospite* spectroscopic measurements.

Time-resolved fluorescence spectra (TRFS) were measured with a time-correlated single-photon counting system at 77 K. The excitation wavelength was 425 nm, at which all pigments in *Prochloron* were simultaneously excited. The time window of measurement was 10 ns with an interval of 2.4 ps/channel, or 100 ns with an interval of 24.4 ps/channel.

Some differences in fluorescence spectra were apparent, especially in the PS (photosystem) I fluorescence region; *Prochloron* in *Diplosoma* sp. and *L. timorense* displayed a more intense PS I signal than *Prochloron* in *T. cyclops* and *T. miniatum*. The TRFS of *Prochloron* indicated that pigment composition and efficiency of excitation energy transfer between antenna proteins in *Prochloron* change with host species. *Prochloron* in *T. cyclops* and *Diplosoma* sp. possess efficient light-harvesting systems, mediated by high efficiency of energy transfer from PS II to PS I. The light harvesting systems of *Prochloron* in *T. miniatum* and *L. timorense* are less efficient. We will discuss relations between these characteristics and light conditions for *Prochloron* cells, such as microenvironments of the ascidians' habitat.

[1] E. Hirose, B.A. Neilan, E.W. Schmidt, A. Murakami, Enigmatic life and evolution of *Prochloron* and related cyanobacteria inhabiting colonial ascidians, in: P.M. Gault, H.J. Marler (Eds.), Handbook on Cyanobacteria, Nova Science Publishers, New York, 2009, pp. 161–189.

## P147: Transcriptional regulators in *Anabaena* sp. PCC 7120

Silvia Picossi, Enrique Flores, and Antonia Herrero

Instituto de Bioquímica Vegetal y Fotosíntesis. CSIC-Universidad de Sevilla. Avda. Américo Vespucio, 49. E-41092 Sevilla. Spain.

In the genome of the nitrogen-fixing cyanobacterium *Anabaena* sp. PCC 7120 there are a number of ORFs encoding transcriptional regulators [1]. There are eight ORFs that code for putative CRP-family proteins and seven encoding putative LysR-type transcriptional regulators, most of which remain to be investigated. The CRP-type proteins are homologous to the *Escherichia coli* regulator CRP, which has a DNA-binding domain at the C-terminal part of the protein and an effector-binding region in the N-terminal part [2]. The best-characterized CRP-type transcriptional factor in cyanobacteria is NtcA, which is the master regulator of nitrogen assimilation genes in these organisms [3]. LysR-type transcriptional regulators have a DNA-binding domain at the N-terminus of the protein, and are usually modulated by small molecules that work as effectors and bind to the C-terminal part of the protein [4]. The best-known LTTRs in cyanobacteria are involved in the regulation of different processes related to carbon assimilation [5, 6] or nitrate assimilation [7]. We are working on the characterization of these transcription factors using different strategies, including chromatin immunoprecipitation (ChIP), to determine their molecular targets and their role in the physiology of *Anabaena*. We have started with an in-depth analysis of NtcA. By performing ChIP followed by sequencing we have determined the complete NtcA regulon at 3 h after nitrogen depletion, in which we have been able to identify the sequences at which NtcA directly binds as a rapid response to nitrogen deprivation. The results obtained showed binding of NtcA to over 2,000 sites throughout the genome. Among the genes to whose promoter regions NtcA binds we have identified several known NtcA-regulated genes, including nitrogen assimilation genes (e.g. *amt4*, *ntcB*, *glnA*) and early heterocyst differentiation genes (e.g. *hetC*, *nrrA*). Additionally, a number of novel sites that will merit further investigation have been identified.

[1] Kaneko, T., Y. Nakamura, C.P. Wolk, et al. (2001) DNA Res. 8: 205-213; 227-253; [2] Korner, H., H.J. Sofia, and W.G. Zumft (2003) FEMS Microbiol. Rev. 27: 559-592. [3] Herrero, A., A.M. Muro-Pastor, and E. Flores (2001) J. Bacteriol. 183: 411-425, [4] Maddocks, S.E. and P.C. Oyston (2008) Microbiology. 154: 3609-3623; [5] Price, G.D., M.R. Badger, F.J. Woodger, and B.M. Long (2008) J. Exp. Bot. 59: 1441-1461; [6] López-Igual, R., S. Picossi, J. López-Garrido, et al. (2012) Environ. Microbiol. 14: 1035-1048; [7] Frías, J.E., E. Flores, and A. Herrero (2000) Mol. Microbiol. 38: 613-625.

## P148: Inactivation of the conserved open reading frame *ycf34* of *Synechocystis* interferes with the photosynthetic electron transport chain

Thomas Wallner<sup>1</sup>, Gábor Bernát<sup>2</sup>, Yoshinori Hagiwara<sup>3</sup>, Edward Reijerse<sup>4</sup>, Nicole Frankenberg-Dinkel<sup>3</sup> and Annegret Wilde<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Molecular Biology, Justus-Liebig-University Giessen, Giessen, Germany; <sup>2</sup>Plant Biochemistry, Ruhr University Bochum, Bochum, Germany; <sup>3</sup>Physiology of Microorganisms, Ruhr University Bochum, Bochum, Germany; <sup>4</sup>Max Planck Institute for Bioinorganic Chemistry, Mülheim an der Ruhr, Germany.

The ancestors of the recent cyanobacteria are the progenitors of the chloroplasts due to an endosymbiotic event that occurred 2 billion years ago. For nearly all proteins that are encoded by chloroplast genomes of algae and plants orthologs exist in cyanobacteria. Although there has been a massive transfer of ancestral cyanobacterial genes to the nucleus, plant and algal chloroplast genomes still contain genes mainly involved in photosynthesis and housekeeping of the organelle. Among these residual genes there exist several open reading frames of unknown function that have been designated *ycf* for hypothetical chloroplast open reading frame. No concluding remarks can be made about these *ycfs* without functional analysis of the resulting gene products.

*Ycf34* is a hypothetical chloroplast open reading frame that is present in the chloroplast genome of several non-green algae. Homologues of *Ycf34* are also encoded in all genomes of cyanobacteria. To evaluate the role of *Ycf34* we have constructed and analysed a cyanobacterial mutant strain. Inactivation of *ycf34* in *Synechocystis* sp. PCC 6803 showed no obvious phenotype under normal light intensity growth conditions.

However, when the cells were grown under very low light intensity they contained less and smaller phycobilisome antennae and showed a strongly retarded growth, suggesting an essential role of the *Ycf34* polypeptide under light limiting conditions. Northern Blot analysis revealed a very weak expression of the phycocyanin operon in the *ycf34* mutant under light limiting growth in contrast to the wild type and to normal light conditions.

Oxygen evolution and P700 measurements showed an impaired electron flow between photosystem II and photosystem I under these conditions which suggest that the impaired antenna size is most likely due to a highly reduced plastoquinone pool which triggers regulation on a transcriptional level. Using a FLAG-tagged *Ycf34* we found that this protein is tightly bound to the thylakoid membranes. UV-Vis and Mössbauer spectroscopy of the recombinant *Ycf34* protein demonstrate the presence of an iron-sulphur cluster.

Since *Ycf34* lacks homology to known iron-sulphur cluster containing proteins, it might constitute a new type of iron-sulphur protein implicated in redox signalling or in optimising the photosynthetic electron transport chain.

## P149: Cyanobacterial instability expressing heterologous pathways

Veronica Carbonell, Fernando Guerrero, Patrik Jones

Bioenergy Group, Department of Biochemistry and Food Chemistry, University of Turku, Tykistökatu 6A, 6krs, 20520 Turku, Finland

The possibility to use cyanobacteria to catalyze the conversion of sunlight, carbon dioxide and water into infrastructure-compatible fuels has aroused the interest of the scientific community. Cyanobacteria can be engineered for biotechnological processes by introducing heterologous genes that modify existing pathways and/or introduce new ones within the host. In many cases, however, the expression of foreign genes can reduce the fitness of the host and result in the evolution of mutant strains with a fitness that is closer to the wild-type and a loss of biotechnological utility. In cyanobacteria many factors might influence the stability of heterologous gene expression. We have taken under consideration five variables that potentially may influence stability: (a) host, (b) expression system, (c) rare codons, (d) terminators, and (e) promoter strength (1, 3). We have used the ethylene forming enzyme (*efe*) from *Pseudomonas syringae* as a model system to test these variables as it only requires a one step reaction to produce an easily measurable end-product (2). This gene has previously been used to transform *Synechococcus elongatus* PCC 7942 through chromosomal integration under control of a strong promoter (4). However, the transformed strains soon lost their ability produce ethylene, the cause was traced to repeatable insert mutations within the opening reading frame of the *efe* gene at sites with the sequence 'CGATG'. Currently, we are reexamining these observations and have prepared three different constructs in a broad host-range plasmid expression system: (i) *oEFE*, the original *efe* gene from *Pseudomonas syringae*, (ii) *syEFE*, a synthetic *efe* gene codon optimized for *Synechocystis* sp. PCC 6803 and (iii) *oEFEΔT*, similar to ii, except lacking a transcription terminator downstream of the *efe* sequence. All the constructs were used to transform *Synechococcus elongatus* PCC 7942 and *Synechocystis* PCC 6803. The stability of the mutants was evaluated by serial evolution with a maximum of seven consecutive cultures. The results demonstrate that correct matching of host selection and choice of expression system has a strong influence since all the constructs were unstable in *Synechococcus elongatus* PCC 7942 and only the two first constructs were stable in *Synechocystis* PCC 6803. As a chromosomal expression system was previously shown to be unstable in *Synechococcus elongatus* PCC 7942 (4), it is not yet possible to distinguish between cause-and-effect with respect to the choice of host and expression system. With the current experimental system, codon optimization did not have any influence on stability whilst the lack of a terminator did. The investigation is continuing.

[1] Dillard JP, Yother J. Analysis of *Streptococcus pneumoniae* sequences cloned into *Escherichia coli*: effect of promoter strength and transcription terminators. [Internet]. *Journal of bacteriology* 1991 Aug;173(16):5105-9. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=208201&tool=pmcentrez&rendertype=abstract>; [2] Hideo Fukuda, Takahira Ogawa, Masato Tazaki, Kazuhiro Nagahama, Takao Fujii ST and YM. Two reactions are simultaneously catalyzed by a single enzyme: the arginine-dependent simultaneous formation of two products, ethylene and succinate, from 2-oxoglutarate by an enzyme from *pseudomonas syringae*. *Biochemical and Biophysical Research Communications* 1992;188(2):483-489; [3] Lindblad P, Lindberg P, Oliveira P, Stensjö K, Heidorn T. Design, Engineering, and Construction of Photosynthetic Microbial Cell Factories for Renewable Solar Fuel Production [Internet]. *Ambio* 2012 Mar;41(S2):163-168. [cited 2012 Mar 28] Available from: <http://www.springerlink.com/index/10.1007/s13280-012-0274-5>; [4] Takahama K, Matsuoka M, Nagahama K, Ogawa T. Construction and analysis of a recombinant cyanobacterium expressing a chromosomally inserted gene for an ethylene-forming enzyme at the *psbAI* locus. [Internet]. *Journal of bioscience and bioengineering* 2003 Jan;95(3):302-5. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16233410>.

## P150: Whole-genome resequencing of *Synechocystis* sp. PCC 6803 substrains

**Yu Kanesaki<sup>1</sup>, Yuh Shiwa<sup>1</sup>, Naoyuki Tajima<sup>2</sup>, Marie Suzuki<sup>3</sup>, Satoru Watanabe<sup>3</sup>, Naoki Sato<sup>2</sup>, Masahiko Ikeuchi<sup>2</sup>, and Hirofumi Yoshikawa<sup>1,3</sup>**

<sup>1</sup>NODAI Genome Research Center, Tokyo University of Agriculture, Tokyo, Japan; <sup>2</sup>Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Japan; <sup>3</sup>Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan

The cyanobacterium, *Synechocystis* sp. PCC 6803, was the first photosynthetic organism whose genome sequence was determined in 1996 (Kazusa strain). It thus plays an important role in basic research on the mechanism, evolution, and molecular genetics of the photosynthetic machinery. There are many substrains such as glucose-tolerant (GT) strain or laboratory strains derived from the original Berkeley strain. To establish reliable genomic sequence data of this cyanobacterium, we performed resequencing of the genomes of three substrains (GT-I, PCC-P, and PCC-N) and compared the data obtained with those of the original Kazusa strain stored in the public database. We found that each substrain has sequence differences some of which are likely to reflect specific mutations that may contribute to its altered phenotype [1]. Our resequence data of the PCC substrains along with the proposed corrections/refinements of the sequence data for the Kazusa strain [1,2] and its derivatives are expected to contribute to investigations of the evolutionary events in the photosynthetic and related systems that have occurred in *Synechocystis*.

[1] Kanesaki Y., Shiwa Y., Tajima N., Suzuki M., Watanabe S., Sato N., Ikeuchi M., and Yoshikawa H. Identification of substrain-specific mutations by massively parallel whole-genome resequencing of *Synechocystis* sp. PCC 6803. *DNA Research* (2012) 19: 67-79; [2] Tajima N., Sato S., Maruyama F., Kaneko T., Sasaki N.V., Kurokawa K., Ohta H., Kanesaki Y., Yoshikawa H., Tabata S., Ikeuchi M., and Sato N. Genomic structure of the cyanobacterium *Synechocystis* sp. PCC 6803 strain GT-S. *DNA Research* (2011) 18: 393-399.

## P151: Characterization of outer membrane in *Synechocystis* sp. PCC 6803, revealed by protein, pigment and lipid analysis

**Lifang Zhang<sup>1</sup>, Tiago Toscano Selão<sup>1</sup>, Eva Selstam<sup>2</sup>, Fang Huang<sup>3</sup>, Birgitta Norling<sup>1</sup>**

<sup>1</sup>Nanyang Technological University, Singapore; <sup>2</sup>Department of Botany, Umeå University, Sweden; <sup>3</sup>Institute of Botany, Chinese Academy of Sciences, China

*Synechocystis* sp. PCC 6803 is a model organism to study oxygenic photosynthesis as well as other metabolic processes. Its three distinct membrane systems also make it ideal to study membrane biogenesis and protein sorting. Many studies have characterized plasma and thylakoid membranes, with a special focus on the proteins present in both of these membranes. However, the outer membrane (OM), the outmost barrier to the surrounding environment, is still underrepresented in these studies.

We have purified OM by a combination of sucrose density gradient centrifugation and two-phase partition and analyzed the protein content by SDS-PAGE and LC-MS/MS. In total, more than 100 proteins were identified, with 58 of them having a predicted N-terminal signal peptide, which makes them possible candidates as OM proteins. Using bioinformatics tools, we have confirmed the identity of 19 out of the 27  $\beta$ -barrel proteins predicted in the *Synechocystis* genome. Seven porins were also newly identified in this work. Of the identified proteins, thirty-nine proteins were predicted to have signal peptides but no predicted  $\beta$ -barrel structure and are either peripheral OM proteins or periplasmic proteins. In contrast to the case in *Escherichia coli*, no lipoproteins could be positively identified in our sample (by LipoP). These results not only update our knowledge of cyanobacterial proteome, but also indicate that insertion of  $\beta$ -barrel proteins into the cyanobacterial outer membrane follows a different process in comparison to other Gram-negative bacteria.

We have furthermore analyzed the pigment composition of OM using HPLC. Most of the pigment present in OM is carotene, with very little chlorophyll *a* being identified. Among all the 8 different types of carotenoids, myxoxanthophyll as well as zeaxanthin are predominant. The *Synechocystis* specific carotenoid synechoxanthin was only found in the outer membrane.

In most Gram-negative bacteria, OM is an asymmetrical bilayer containing lipopolysaccharide (LPS) exclusively in the outer leaflet and phospholipids in the inner leaflet. However, unlike most Gram-negative bacteria, cyanobacterial membranes contain a large proportion of sugar and sulpholipids. 2D-TLC was carried out in order to separate and identify major lipid classes in OM. Our results show that MGDG, SQDG and DGDG are the major lipids in OM, as is the case for plasma and thylakoid membranes. However, unlike these, OM is the only membrane containing PE, PI and PA, raising further questions regarding OM biogenesis.

## P152: Effects of CO<sub>2</sub> on flash-induced chlorophyll fluorescence decay in *Thermosynechococcus elongatus*

**Zsuzsanna Deák and Imre Vass**

Institute of Plant Biology, Biological Research Center, Szeged, Hungary

Increase and subsequent decay of flash-induced chlorophyll (Chl) fluorescence provides a useful tool in Photosystem II (PSII) electron transport studies. Excitation of dark adapted photosynthetic samples with a short, saturating light pulse induces the reduction of Q<sub>A</sub>, the first quinone acceptor of PSII, which results in fluorescence increase from initial F<sub>0</sub> level to the F<sub>m</sub> maximal level. Following flash excitation Q<sub>A</sub><sup>-</sup> is reoxidized via different pathways and typically results in a decrease of Chl fluorescence yield back to the F<sub>0</sub> level.

Interestingly, in intact cells of the thermophilic cyanobacterium *Thermosynechococcus elongatus* a novel feature of the flash-induced Chl fluorescence can be observed. Instead of a monotonically decreasing behavior the relaxation curve shows a drop with a minimum around 100 ms followed by an increase up to few seconds, resulting in a transient oscillation of the fluorescence yield [1]. This unusual characteristic of the flash-induced fluorescence decay is abolished in the presence of electron transport inhibitors (DCMU, DBMIB), or the artificial electron acceptor (DMBQ), indicating that changes in the redox-state of the PQ-pool, and free electron movement through it are essential in the appearance of the oscillation [1].

In cyanobacteria the redox-state of the PQ-pool is determined by the simultaneous activity of photosynthetic and respiratory electron transport chains. The NAD(P)H-quinone oxidoreductase (NDH-1) plays an important role in the respiration as well as it is involved in cyclic electron flow around PSI and CO<sub>2</sub> uptake [2]. In cyanobacteria CO<sub>2</sub> concentrating mechanism (CCM) functions to elevate CO<sub>2</sub> concentration around the active site of Rubisco, in the carboxisomes, to compensate for the low affinity of their Rubisco for CO<sub>2</sub> [3]. At higher temperatures Rubisco decreases its affinity to CO<sub>2</sub>, while its oxygenase activity is increased. Thus cyanobacteria actively photosynthesising at elevated temperatures may be expected to require more effective CCM [4]. It was also shown in the literature, that NDH-1 complex and NDH-1 mediated cyclic electron transport are stimulated under low CO<sub>2</sub> [5]. To investigate the effect of CO<sub>2</sub> concentration on the oscillating flash-induced fluorescence decay *Thermosynechococcus elongatus* cells routinely grown at 40°C were cultured under low (0.1%) and also under high (1%) CO<sub>2</sub>. The effects of CO<sub>2</sub> concentration on the special oscillating feature of the flash-induced fluorescence decay will be presented and discussed in details.

[1] Deák Zs, Vass I (2008) In: Allen JF, Gantt E, Golbeck JH and Osmond B (eds.) Photosynthesis. Energy from the Sun. Springer, pp 573-576; [2] Battchikova N, Eisenhut M, Aro E-M (2011) Biochim. Biophys. Acta 1807, 935-944; [3] Ogawa T, Kaplan A (2003) Photosynth. Res. 77, 105-115; [4] Badger MR, Price GD, Long BM, Woodger FJ (2006) J. Exp. Bot. 57, 249-265, [5] Deng Y, Ye J, Mi H (2003) Plant Cell Physiol. 44, 534-540.

## P153: Characterization of the novel monogalactosyldiacylglycerol (MGDG) synthase, found in green sulfur bacteria, reveals mechanisms of chlorosome biogenesis

**Shinji Masuda<sup>1</sup>, Jiro Harada<sup>2</sup>, Masato Noguchi<sup>2</sup>, and Hiroyuki Ohta<sup>1</sup>**

<sup>1</sup>Center for Biological Resources and Informatics, Tokyo Institute of Technology, Japan; <sup>2</sup>Department of Medical Biochemistry, Kurume University School of Medicine, Japan

Chlorosome is a light-harvesting complex found in some photosynthetic bacteria classified to *Chlorobi*, *Chloroflexi* and *Acidobacteria* phyla. Chlorosome is the largest and the most efficient antenna systems in nature, which allows the bacteria to perform photosynthesis under extraordinarily low-light conditions. Chlorosome is constructed from carotenoids, quinones, and hundreds of thousands of bacteriochlorophyll molecules inside the monolayer membrane vesicle containing a large amount of galactolipids (~60 % of polar lipids) such as MGDG [1]. Although protein and lipid compositions as well as the organization of bacteriochlorophylls in chlorosomes have been well documented, how this unique light-harvesting system is generated in native cells remains poorly understood [2,3].

We previously identified the novel MGDG synthase from the green sulfur bacterium, *Chlorobaculum tepidum* [4]. MgdA shows MGDG synthase activity by use of UDP-galactose as a substrate. Western blot analysis indicated that MgdA localizes on the cytoplasmic membrane in *C. tepidum*. *mgdA* is essential for this bacterium; only heterozygous *mgdA* mutant could be isolated. The mutant showed reduced levels of *mgdA* mRNA, indicating that they are actually *mgdA*-knockdown mutants. The knockdown mutation affects the assembly of chlorosomes, suggesting that MGDG synthesis by MgdA in cytoplasmic membrane is important for chlorosome construction. Detailed mechanisms of chlorosome biogenesis will be discussed.

[1] Sørensen et al. (2008) Photosynth. Res. 95: 191-196; [2] Hohmann-Marriott & Blankenship (2007) FEBS Lett. 581: 800-803; [3] Pederson et al. (2010) Photosynth. Res. 104: 233-243; [4] Masuda et al. (2011) Plant Cell 23: 2644-2658.

## P154: A gene regulation mechanism allowing for the synthesis of two “ferredoxin:NADP oxidoreductase” isoforms from a single gene in the cyanobacterium *Synechocystis* sp. PCC 6803

A. Omairi Nasser<sup>1</sup>, C. Galmozzi<sup>2</sup> and M.I. Muro-Pastor<sup>2</sup> and G. Ajlani<sup>1</sup>

<sup>1</sup>Institut de Biologie et de Technologie de Saclay, CNRS and CEA, F-91191 Gif-sur-Yvette, France; <sup>2</sup>Instituto de Bioquímica Vegetal y Fotosíntesis, CSIC-Universidad de Sevilla, Américo Vespucio 49, E-41092 Sevilla, Spain.

Ferredoxin:NADP oxidoreductase (FNR), encoded by the *petH* gene, provides NADPH for CO<sub>2</sub> fixation in photoautotrophic cells and oxidizes NADPH in heterotrophic cells. Whereas there is only one *petH* copy in the cyanobacterium *Synechocystis* sp. PCC6803, two FNR isoforms accumulate (FNR<sub>L</sub> and FNR<sub>S</sub>). It was proposed that FNR<sub>L</sub> fulfills functions in linear electron transport while FNR<sub>S</sub> is involved in cyclic-electron transport and respiration. FNR<sub>S</sub> was shown to be the product of an internal translation initiation within the FNR<sub>L</sub> open-reading frame.

In this work, we analyzed the mechanism by which *petH* translation leads to the accumulation of either one of the FNR isoforms. Basically, we showed that *petH* 5'noncoding region was essential for FNR<sub>S</sub> accumulation. Deletions in the 5'noncoding region suggested that each isoform is produced from a specific mRNA. 5'-end mapping of the *petH* transcripts confirmed this fact and showed that under standard conditions -when FNR<sub>L</sub> accumulates- two mRNAs carrying similar leaders (32 and 53 bases) are transcribed; while under nitrogen starvation -when FNR<sub>S</sub> accumulates-, an mRNA carrying a longer leader (126 bases) is transcribed.

EMSA showed that the global nitrogen regulator NtcA binds to the upstream region of *petH*. Mutagenesis of a putative NtcA-binding site resulted in the abolition of FNR<sub>S</sub> accumulation. Thus we identified an NtcA binding site 42 nucleotides upstream from the long transcript 5' end.

Transcriptional fusions of the *E. coli lac* promoter to different *petH* leader sequences, showed that translation regulation does not require a specific factor present in *Synechocystis* sp. only; but rather to a spontaneously occurring secondary structure, adopted by the longer leader. Such a structure could activate FNR<sub>S</sub> translation initiation and prevent that of FNR<sub>L</sub>.

Hence, we have uncovered a novel gene-regulation mechanism by which two isoforms are produced from a single gene in a cyanobacterium.

## P155: A wide diversity of proteobacterial plasmids can conjugate with cyanobacteria

David Encinas Pisa<sup>1</sup>, M<sup>a</sup> Pilar Garcillán Barcia<sup>1</sup>, Luis Delaye<sup>2</sup>, Andrés Moya<sup>2</sup> and Fernando de la Cruz<sup>1</sup>

<sup>1</sup>Departamento de Biología Molecular.Universidad de Cantabria. Instituto de Biomedicina y Biotecnología de Cantabria IBBTEC (UC-IDICAN-CSIC). Santander. <sup>2</sup>Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Valencia.

Comparative genomic analysis of the *Synechococcus elongatus* chromosome showed that a majority of *Synechococcus* proteins of xenologous origin (253 proteins) probably originated from the phylum proteobacteria. This suggested that functional mechanisms must exist that genetically bridges cyanobacteria and proteobacteria. One potential mechanism could be conjugation. The aim of this work was therefore to analyze if proteobacterial plasmids were in general capable of transmitting plasmids from proteobacteria to cyanobacteria by conjugation.

For this purpose, we developed a series of BioBrick-compatible shuttle vectors capable of replicate both in *E. coli* and cyanobacteria. Five proteobacterial conjugative plasmids (R388, RP4, pKM101, R64 and pOX38) that use widely diverse conjugative systems, were tested for their ability (a) to conjugate themselves, and (b) to mobilize pDEP006, a synthetic plasmid vector based on the replicon of the endogenous *Synechococcus* plasmid pANL from *E.coli* to cyanobacteria. All five plasmids were able to introduce their antibiotic resistance genes in *Synechococcus*, although they were all highly unstable and were lost after subculturing. On the other hand, all five plasmids mobilized pDEP006 and stable transconjugants could be repeatedly subcultured. RP4-derived pDEP006 transconjugants were analyzed by extracting DNA and transforming back to *E. coli*. The pDEP006 restriction pattern was unaltered. It is difficult to measure conjugation rates in our experiments; just judging from the number of transconjugants, widely different rates are inferred. In this respect, an important part of our work was also to simplify and optimize the existing conjugation protocol to cyanobacteria. As a result, the protocol was reduced from about 20 days to just 10 days, with a significant increase in the number of real transconjugants. The new protocol should be a useful tool to accelerate genetic manipulations of cyanobacteria.



## P156: Understanding the mechanisms for alkane synthesis in cyanobacteria

**Francy El Souki<sup>1</sup>, Andras Pasztor<sup>1</sup>, Laura Holtmann<sup>2</sup>, Guy Hanke<sup>2</sup>, Patrik R. Jones<sup>1</sup>**

<sup>1</sup>Molecular Plant Biology, Department of Biochemistry and Food Chemistry, University of Turku, Finland; <sup>2</sup>University of Osnabruck, Division of Plant Physiology, Germany

There is a current need for finding ways for production of renewable energy, the natural pathway of cyanobacteria to produce alkanes represents a potential alternative. This work aims to understand the alkane synthesis in Cyanobacteria, specifically to characterize the enzyme involved in the last step of alkane biosynthesis: decarbonylase (AD). Recent report in cyanobacteria identifies two open reading frames that code for the decarbonylase and acyl-ACP reductase that are necessary to catalyze the last step in alkane production [1]. Furthermore, the *in vitro* characterization of the AD suggests that a reducing environment is needed [2]. Based on these findings we were interested to explore the role of ferredoxin as an *in vivo* cofactor in a heterologous system. In order to achieve this objective, AD from *Synechosystis* sp. PCC6803 and *Nostoc punctiforme* PCC73102 (NP) were expressed in *E. coli* BL21(DE3) together with an acyl-ACP reductase (AAR) from *Synechosystis* sp. PCC6803. Three ferredoxin-encoding genes from *Synechosystis* sp. PCC6803 were co-expressed in this system in order to test their role as native electron donor; alkane production was measured using gas chromatography mass spectrometry, including standards of alkanes and alcohols. The novel products pentadecane, 8-heptadecene, heptadecane, hexadecanol and oleyl alcohol, were not present in *E. coli* BL21(DE3) without plasmids. The accumulation of alkanes, but not alcohol, was enhanced by co-expression of ferredoxin, however, not all ferredoxins resulted in the same increase of alkane synthesis. Surprisingly, the recombinant expression of AAR from *Synechosystis* together with AD from NP resulted in a low production of alkanes, in contrast to the total *in vitro* activity. Together, this suggests that a degree of compatibility exists between the three proteins from the same cyanobacteria species and that correct matching of pathway components is needed to maximize pathway flux. This may be explained by several factors including physical interaction between components and varying substrate specificities.

[1] Schirmer A., Rude M.A., Li X., Popova E., Stephen B. del Cardayre S. B. 2010. Microbial biosynthesis of alkanes. *Science* 329: 559 - 562; [2] Debasis D., Eser B.E., Han J., Sciore A., Marsh E.N.G. (2011). Oxygen-Independent Decarbonylation of Aldehydes by Cyanobacterial Aldehyde Decarbonylase: A New Reaction of Diiron Enzymes. *Angewandte Chemie* 50 (31), 7148-7152.

## P157: CyanoExpress: A Comprehensive Database for Evaluating the Transcriptional Response in *Synechocystis*

**Miguel A. Hernández-Prieto, and Matthias E. Futschik**

Centre for Molecular and Structural Biomedicine, University of Algarve, 8005-139 Faro, Portugal

The cyanobacterium *Synechocystis* sp. PCC6803 is the most extensively studied photosynthetic prokaryote. During recent years, numerous genome-wide expression measurements have been carried out for a large variety of environmental and genetic perturbations. In fact, over 600 individual microarray measurements are stored in public repositories. Undoubtedly, this massive data set can provide us with a plethora of new insights into the regulation of single genes, as well as into the coordination of cellular processes.

At present, researchers seeking to utilize the accumulated data for *Synechocystis* face several difficulties, since these publically accessible data comprise different microarray platforms, data formats, as well as data processing approaches. Hence, these data must first be integrated. This integration is both an error-prone and time-consuming task. To assist researchers to overcome these difficulties, we have developed a web-accessible database termed CyanoExpress [1]. This new database enables users to browse expression data from many individual experiments in an intuitive way. The integrated data stored in CyanoExpress contains expression data for 3 165 protein-coding genes, derived from 651 microarrays, used in 28 individual experiments covering 166 different conditions (i.e. environmental or genetic perturbations). Special care was taken in the curation and pre-processing of the microarray data to avoid database errors and to obtain a large set of distinct expression patterns.

CyanoExpress can be used in several ways: i) Users can simply input single genes to retrieve co-expressed genes and ascribe potential functional partners; ii) Users can inspect select functionally-related genes (for instance, genes associated with photosynthesis) and inspect their expression across the different environmental perturbations; and iii) Users can zoom into cluster structures, which we detected in a meta-analysis, and identify strong co-regulation patterns [2]. CyanoExpress is based on the open-source web server application GeneExplorer [3] (Rees, et al. 2004), and implemented using CGI, JavaScript and HTML. CyanoExpress is freely accessible by any web browser at <http://CyanoExpress.sysbiolab.eu>.

[1] M. A. Hernández-Prieto, and M. E. Futschik. "CyanoExpress: A Comprehensive Database for Interrogating the Transcriptional Response in *Synechocystis*". Manuscript in preparation; [2] M. A. Hernández-Prieto, C. Steglich, J. Varela, W. Hess and M. Futschik. "The transcriptional landscape of the cyanobacterium *Synechocystis*". Submitted manuscript; [3] Rees, C., J. Demeter, et al. (2004). "GeneExplorer: an interactive web application for microarray data visualization and analysis" *BMC Bioinformatics* 5(1): 141.



## P158: Simultaneous profiling of coding and non-coding RNAs from *Synechocystis* under iron-limiting conditions

**Miguel A. Hernández-Prieto<sup>1</sup>, Verena Schön<sup>2</sup>, Claudia Steglich<sup>2</sup>, Wolfgang Hess<sup>2</sup>, Matthias E. Futschik<sup>1</sup>**

<sup>1</sup>Centre for Molecular and Structural Biomedicine, University of Algarve, 8005-139 Faro, Portugal; <sup>2</sup>Faculty of Biology, University of Freiburg, 79104 Freiburg, Germany

For many decades, RNA levels in the cell were thought to be primarily determined by transcription factors. In recent years, this view has been challenged by the discovery of a large number of non-coding RNAs in cyanobacteria using novel sequencing technologies. These non-coding RNAs appear to participate at several levels of gene regulation, including transcription [1]. The unexpectedly large number of putative non-coding RNAs points to a far more complex regulatory system underlying gene expression than previously thought. Our study of iron homeostasis in *Synechocystis* further supports this conjecture.

Iron is an essential cofactor in many metabolic reactions. Although it is the fourth most common element in the earth's crust, iron is a limiting factor for aquatic microorganisms due to its insolubility in the presence of oxygen at neutral pH. Cyanobacteria have therefore developed several strategies to increase intracellular Fe<sup>2+</sup>, including synthesis of specific transporters and secretion of ferric ion chelating agents. However, the concentration of intracellular iron must be kept under strict control due to its high reactivity, producing reactive oxygen species through Fenton-type reactions. Thus, mechanisms controlling iron homeostasis must respond not only to changes in extracellular conditions, but also intracellular conditions. To gain insights into the molecular details of iron homeostasis in cyanobacteria, we measured time-resolved gene expression changes after iron depletion in *Synechocystis* sp. PCC 66803, using a comprehensive microarray platform. In contrast to earlier studies, we utilized a customized oligonucleotide microarray, including both coding and non-coding genes. In total, almost 600 protein-coding genes were differentially expressed during the first 72h. Many of these genes are directly or indirectly associated with iron transport, photosynthesis and ATP synthesis. Our results correlate well with previously published expression studies of *Synechocystis* sp. PCC 6803 upon iron perturbation.

In addition to the expression changes in coding genes, we found that a strikingly large number of non-coding RNAs (N=647) showed significant changes in expression under iron limiting conditions. Through statistical and clustering analyses, we identified several non-coding RNAs that may function as key regulatory elements in iron homeostasis, in addition to the iron-sensing transcription factor FurA (Ferric uptake regulator).

The genome-wide profiling presented here provides an unprecedented picture of the coordinated action of coding and non-coding RNAs to maintain iron homeostasis in cyanobacteria. It serves as a prime example of the complex interplay of various types of components in regulation of iron homeostasis in prokaryotes.

[1] Mitschke, J., J. Georg, et al. (2011). PNAS 108(5): 2124-2129.

## P159: Regulation mechanism of DNA replication in cyanobacteria *Synechococcus elongatus* PCC7942

**Ryudo Ohbayashi<sup>1</sup>, Satoru Watanabe<sup>1</sup>, Yu Kanesaki<sup>2</sup>, Rei Narikawa<sup>3</sup>, Taku Chibazakura<sup>1</sup>, Masahiko Ikeuchi<sup>3</sup> and Hirofumi Yoshikawa<sup>1</sup>**

<sup>1</sup>Department of Bioscience, Tokyo University of Agriculture, Tokyo, Japan; <sup>2</sup>Genome Research Center, NODAI Research Institute, Tokyo University of Agriculture, Tokyo, Japan; <sup>3</sup>Department of Life Sciences, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan

Fresh water cyanobacteria *Synechococcus elongatus* PCC7942 (hereafter *S. 7942*) exhibit light-dependent cell growth. In a previous study, we showed that DNA replication also depends on light irradiation [1]. To reveal the regulation mechanism of the DNA replication in *S. 7942*, we investigated the effects of inhibitors.

We analyzed the BrdU incorporation activity using the dark-synchronized *S. 7942* culture. When the culture was transferred to the light condition, the BrdU was incorporated into their genome. The BrdU uptake was clearly inhibited by the addition of the Rifampicin and the Chloramphenicol. These results indicate that the replication initiation depends on *de novo* protein synthesis. DCMU and DBMIB clearly blocked not only the BrdU incorporation but also the expression of RpoD1. These results suggest that the activation of photosynthetic electron transfer is the cue for both *de novo* protein synthesis and DNA replication initiation. In addition, the DNA replication progression, which was evaluated by the BrdU uptake activity 9 h after the transfer, was inhibited by the addition of DBMIB, but not the DCMU. A possible model of DNA replication in *S. 7942* will be discussed.

[1] Watanabe S., Ohbayashi R., Shiwa Y., Noda A., Kanesaki Y., Chibazakura T., and Yoshikawa H., Light-dependent and asynchronous replication of cyanobacterial multi-copy chromosomes. Mol. Microbiol. (2012) 83: 856-865

## P160: Unique acclimation processes of the chlorophyll *d* containing cyanobacterium, *Acaryochloris marina*

Éva Kiss<sup>1</sup>, Péter B. Kós<sup>1</sup>, Min Chen<sup>2</sup>, Imre Vass<sup>1</sup>

<sup>1</sup>Institute of Plant Biology, Biological Research Center, Szeged, Hungary; <sup>2</sup>School of Biological Sciences, University of Sydney, Sydney, NSW 2006, Australia.

Cyanobacteria are among the most adaptive photosynthetic organisms on Earth. *Acaryochloris marina* with its exceptionally large and complex genome is likely to be one of the most versatile cyanobacterial species, when it comes to acclimation to different light conditions. In the present study the acclimation of *Acaryochloris marina* was followed by chlorophyll fluorescence, oxygen evolution, absorbance measurement as well as expression assessments of the genes encoding the D1/D2 core heterodimer and the cytochrome b559 subunits of Photosystem II reaction center.

The chromatic-acclimation of *Acaryochloris marina* to low light intensities results in a red shift in the *in vivo* absorption peak of chlorophyll *d* suggesting that adjustment of the light harvesting system to poor availability of accessible light involves the chlorophyll *d* containing Pcb inner antennas. *Acaryochloris marina* was found to synthesize more Photosystem II centers, while decreasing its phycobiliprotein content when adapting to far red light. Both, far red, and low intensity illumination caused the oxidation of the plastoquinone pool, indicating a strong oxidizing force on the pool, which was also observed during dark adaptation. According to the present study the remarkable ability of *Acaryochloris marina* to acclimate to different light conditions is also related to its unique photosynthetic gene arsenal including 3 *psbA* and 3 *psbD* genes, which encode 2 different isoforms of the D1 and D2 subunits of the core heterodimer of Photosystem II, respectively. A significant difference in the relative amount of the *psbE* and *psbF* transcripts encoding the alpha and beta subunits of cytochrome b559 was found. These data indicates that in contrast to the already characterized Photosystem II complexes in which the protein backbone of cytochrome b559 is made up by an alpha-beta heterodimer the Photosystem II of *Acaryochloris marina* is likely to contain an alpha-alpha homodimer of the cytochrome b559 subunits [1].

[1] É. Kiss, P. B. Kós, I. Vass, (2012) A unique regulation of the expression of the *psbA*, *psbD*, and *psbE* genes, encoding the D1, D2 and cytochrome b559 subunits of the Photosystem II complex in the chlorophyll *d* containing cyanobacterium *Acaryochloris marina* BBA Bioenergetics doi:10.1016/j.bbabi.2012.04.010.

## P161: A new player in the iron-regulatory network of cyanobacteria

Verena Schön<sup>1</sup>, Jens Georg<sup>1</sup>, Heiko Lokstein<sup>1,2</sup>, Claudia Steglich<sup>1</sup> and Wolfgang R. Hess<sup>1</sup>

<sup>1</sup>Faculty of Biology, University of Freiburg, Freiburg, Germany; <sup>2</sup>current address: Inst. of Molecular, Cell and Systems Biology, University of Glasgow, Glasgow, UK

Small regulatory RNAs occur in all domains of life. Recently we have identified the sRNA SyR22 (Ncl1600) in the course of a transcriptome analysis of the unicellular model cyanobacterium *Synechocystis* sp. PCC6803 [1], which appears to be conserved also in several other uni- and multicellular cyanobacteria. RNA profiling using custom-made microarrays covering all transcripts in *Synechocystis* revealed that the expression of SyR22 becomes induced several orders of magnitude upon iron limiting conditions. Iron is an important cofactor for electron transport systems and enzymes involved in redox reactions. A knockout mutant of SyR22 ( $\Delta$ SyR22) was found to be less viable under iron limited conditions suggesting an important role of SyR22 in the iron stress response. Analyzing the photosynthetic characteristics of the  $\Delta$ SyR22 mutant revealed lack of the typical blue shift of photosystem I (PSI) absorption, of the characteristic changes in 77 K fluorescence emission spectra and of the non-photochemical quenching response at room temperature, which occur due to accumulation of the IsiA protein under these conditions. IsiA, the iron stress-induced protein A, becomes expressed under iron starvation in many cyanobacteria. It absorbs light upon chlorophyll binding and forms giant multimeric rings, which contribute to the protection of the photosynthetic apparatus and compensate for the reduction in the number of PSI complexes under iron limitation. Indeed, in wild-type cells, the induction and repression kinetics under iron stress and following the re-addition of iron are virtually identical for SyR22 and *isiA*. In contrast, in  $\Delta$ SyR22 cells IsiA protein is accumulated in a delayed fashion and not to the same amount as observed in wild-type cells. Detailed mutational analyses revealed a small sequence element in the *isiA* 5'UTR that is recognized by SyR22 and through which a positive effect on the translation and stability of the *isiA* mRNA is mediated. The expression of *isiA* in *Synechocystis* is also controlled by another RNA regulator, the small *cis*-antisense RNA IsrR in a negative way [2]. We conclude that *isiA* is the first known example of a bacterial gene in which a trans-acting sRNA (SyR22) and a *cis*-antisense RNA (IsrR) jointly control gene expression. In addition, phenotypic characterization and microarray analyses of the  $\Delta$ SyR22 and SyR22 overexpressor mutants suggested the involvement of SyR22 in the control of several more genes. We conclude that SyR22 is an only 68 nt long riboregulator of possibly central importance in cyanobacteria.

[1] Mitschke J., et al. (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC 6803. Proc. Natl. Acad. Sci. USA 108, 2124-2129; [2] Dühring U., et al. (2006): An internal antisense RNA regulates expression of the photosynthesis gene *isiA*. Proc. Natl. Acad. Sci. USA, 103, 7054-7058.

## P162: Coping without Fur: global regulation of iron homeostasis by the heme synthesis regulator HbrL in *Rhodobacter capsulatus*

**Sébastien Zappa and Carl E. Bauer**

Indiana University, Biochemistry Department, Simon Hall 313, 212 Hawthorne Dr, Bloomington, IN 47405, U.S.A.

The best well-characterized iron regulator in bacteria is Fur (Ferric uptake regulator) found in a broad range of proteobacteria species as well as bacilli and cyanobacteria.  $\alpha$ -proteobacteria are of particular interest as some branches have maintained the Fur regulation as well as the inclusion of additional iron regulators Irr and RirA. The DNA binding activity of Fur involves Fe(II) as co-regulator whereas Irr senses the intracellular level of iron through the status of heme synthesis and RirA senses iron as a function of the status of Fe-S centers. *Rhodobacter capsulatus* represents a unique example among the  $\alpha$ -proteobacteria as it is the only species known to have lost Fur and have not gained RirA. In this species, Irr was presumed to act as the master regulator of iron homeostasis, however this appears not to be the case as an in-frame *irr* deletion mutant has the same growth characteristics in iron-repleted and iron-depleted media. Instead, we show that HbrL, previously identified as a heme synthesis repressor, has a central role in the regulation of iron in this species. Unlike wild-type *R. capsulatus* cells, an  $\Delta hbrL$  strain was unable to grow in iron-limited medium. Moreover, sequence analysis of suppressor strains revealed the presence of mutations in the iron uptake genes Fe(III)-siderophore transport and Fe(II) permease. Gene expression studies revealed that HbrL is involved in the overexpression of a number of Fe systems including: i) the ferrous iron uptake systems Feo1, Feo2 and to a lesser extent EfeUOB; ii) seven ferric-siderophore uptake systems as well as a ferric-ABC transporter; iii) a heme uptake system; iv) all nine heme synthesis genes. Interestingly, the Fe storage bacterioferritin and an Fe(II) efflux pump were found not to be part of the HbrL regulon. A laboratory strain collection was also screened for the presence/absence of *hbrL* in *R. capsulatus* isolates. Out of eight tested strains, three strains that form a distinct 16S clade were found to lack *hbrL* and interestingly, two of these *hbrL*-less strains showed reduced tolerance to iron limitation. As a conclusion, the gain of *hbrL* is recent and, along with the loss of *fur*, seems to be specific to *R. capsulatus* in  $\alpha$ -proteobacteria. Thus, HbrL appears as a global regulator capable of repressing heme synthesis in the presence of exogenous heme as well as activating the expression of iron and heme uptake systems under iron limited conditions. The role of HbrL as both a master regulator of iron, and its cognate tetrapyrrole heme, homeostasis appears novel and in this species also raises the question of the role of Irr.

## P163: Reconstitution of Bacteriochlorophyll $\alpha$ Derivatives Esterified with Different Hydrocarbon Chains into Light-harvesting Complexes 2 of Purple Photosynthetic Bacteria

**Yoshitaka Saga, Kenji Kawamura, and Takeru Sakamoto**

Department of Chemistry, Faculty of Science and Engineering, Kinki University, Higashi-Osaka, Japan

In the light-harvesting complex 2 (LH2) of purple photosynthetic bacteria, bacteriochlorophyll (BChl) *a* molecules are organized in a circular form to capture light-energy and transfer it to the reaction center *via* LH1 complexes with high efficiency. Such an excellent arrangement of BChl *a* in LH2 has attracted considerable attention and will be useful for developments of photoactive nano-devices. Reconstitution of unnatural pigments into LH2 proteins is one of the promising strategies for construction of photo-functional nano-biomaterials based on photosynthetic supramolecular structures. We examine reconstitution of BChl *a* derivatives esterified with various hydrocarbon chains into the B800 sites of LH2 toward construction of artificial photosynthetic complexes by combination of LH2 proteins with unnatural BChl *a* derivatives possessing functional moieties at the terminus of the esterifying chains. Some Zn-BChl *a* derivatives, which possessed different hydrocarbon chains at the 17-propionate residue, were synthesized from natural BChl *a* extracted from the purple photosynthetic bacterium *Rhodobacter sphaeroides*. LH2 was also obtained from *Rhodobacter sphaeroides*. Removal of B800 BChl *a* from LH2 complexes and subsequent incorporation of Zn-BChl *a* derivatives into these sites were performed according to a previous report [1]. Our reconstitution studies suggest that the B800 sites of LH2 can accept not only natural BChl *a* with a phytol chain but also BChl *a* derivatives possessing other long hydrocarbon chains.

[1] N. J. Fraser, P. J. Dominy, B. Ücker, I. Simonin, H. Scheer, R. J. Cogdell, *Biochemistry* 38, 9684-9692 (1999).



# **POSTER>SESSION V**

## **BIOREMEDIATION, SECONDARY METABOLITES AND OTHER APPLIED ASPECTS**





## POSTERS> V. BIOREMEDIATION, SECONDARY METABOLITES AND OTHER APPLIED ASPECTS

### P164: A novel protease inhibitor from the terrestrial cyanobacterium *Nostoc* sp.

**Kapusčík A.<sup>1,2</sup>, Kuzma M.<sup>3</sup>, Novák P.<sup>3,4</sup>, Hrouzek P.<sup>1,2</sup>, Kopecký J.<sup>1,2</sup>, Pflüger M.<sup>5</sup>, Hundsberger H.<sup>5</sup>**

<sup>1</sup> Institute of Microbiology, Department of Phototrophic Microorganisms – ALGATECH, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic; <sup>2</sup> Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic; <sup>3</sup> Institute of Microbiology, Academy of Sciences of the Czech Republic, Laboratory of Molecular Structure Characterization, Prague, Czech Republic; <sup>4</sup> Department of Biochemistry, Faculty of Science, Charles University, Praha, Czech Republic; <sup>5</sup> Medical and Pharmaceutical Biotechnology, IMC University of Applied Sciences, Krems, Austria.

Aeruginosins, produced by cyanobacteria, mainly *Microcystis* and *Planxtothrix*, are known inhibitors of serine proteases [1]. The relatively small linear peptides contain at the N-terminus a derivative of hydroxyphenyl lactic acid (Hpla), a variable proteinogenic amino acid residue, 2-carboxy-6-hydroxyoctahydroindole (Choi) and arginine derivative side chain at the C-terminus, moreover the structure can undergo various modifications. Biosynthesis of aeruginosins starts with activation of phenylpyruvate which is reduced to Hpla by hybride NRPS/PKS enzyme [2].

A new trypsin and elastase inhibitor of aeruginosins structure was isolated from methanol-water extract from *Nostoc* sp. Lukešová 30/93 collected in Krušné Hory in the Czech Republic. Homonuclear and heteronuclear NMR techniques as well as HRESIMS determined the structure of the new compound, that differs from the other known aeruginosins in presence of the uronic acid and hexanoic acid moieties attached to the Choi substituent.

Subsequent bioactivity screening on AlphaLISA assay<sup>TM</sup> revealed, that the new aeruginosin significantly decreases the level of interleukin-8 in activated human lung microvascular endothelial cells, thus it can be of importance as an anti-inflammatory agent.

[1] Welker M, von Dörhen H. (2006): Cyanobacterial peptides – Nature's own combinatorial biosynthesis. *FEMS Microbiol. Rev.* 30; 530-563

[2] Ishida K, Welker M, Christiansen G, Cadel-Six S, Bouchier C, Dittmann E, Hertweck C, Tandeau de Marsac N. (2009): Plasticity and evolution of aeruginosin biosynthesis in cyanobacteria. *Appl. and Environ. Microbiol.* 75; 2017-2026  
(e-mail: olak@alga.cz; phone +420-384-340465; fax +420 384 340415)

### P165: Effect of phosphorus on microcystin production and *mcyE* expression in *Anabaena* sp. 90

**Anne Rantala-Ylinen, Hanna Sipari, Julia Österholm, Matti Wahlsten, and Kaarina Sivonen**

Department of Food and Environmental Sciences, Division of Microbiology, University of Finland, Helsinki, Finland

The most common toxins produced by cyanobacteria, e.g. *Anabaena*, *Microcystis*, and *Planktothrix*, are the hepatotoxic microcystins. They pose a risk for water users, especially when cyanobacteria form dense mass occurrences (blooms) in water bodies. The environmental factors, e.g. key nutrients (P, N), temperature, and light affect the growth and formation of blooms. These environmental factors have also been associated with higher microcystin concentrations in water and with increased occurrence probability of microcystin-producing cyanobacteria. Microcystins are synthesized by large multi-enzyme complexes called microcystin synthetases encoded by microcystin synthetase (*mcy*) genes. The *mcy* genes are constitutively expressed. However, light, nutrient availability and growth phase have been shown to affect transcription of *mcy* genes in *Microcystis* and *Planktothrix*. Here, we studied effects of P concentration on microcystin production and expression of the *mcyE* gene in an axenic cyanobacterial strain *Anabaena* sp. 90. First, the strain was cultured for one week in media containing high P concentration (5.5 mg/l) and transferred to media containing no P for one week in order to drain its intracellular P storage. The culture was then divided and transferred to growth media containing either high or low P concentration (0.1 or 0.05 mg/ml). All the cultures were sampled on days 2, 4, 6, 8, and 10 for determination of optical density by spectrophotometer, microcystin analysis by LC-MS, and RNA extraction and subsequent determination of *mcyE* transcription levels by reverse transcription - quantitative PCR. Transcription of the *mcyE* gene was normalized with transcription levels of three reference genes.

P concentration had a clear effect on both growth and microcystin concentrations. *Anabaena* sp. 90 grew slower and produced decreasing amounts of microcystins in low P conditions. On the last day of the experiment, microcystin concentration was about 20% of the concentration detected in cells grown in high P. In all cultures, similar trend in *mcyE* expression was detected. The expression decreased after P starvation and increased after transfer back to P-containing media and started to decline towards the end of the experiment. In general, highest expression was observed at days 4 and 6. Surprisingly, the increase in transcription was seen both in high and low P conditions. The results suggest that regulation of microcystin production might occur post-transcriptionally.

## P166: Small peptide profiling of *Nostoc* spp. isolated from plant symbioses

**Anton Liaimer, Janne Stangeland**

Molecular Environments Group, Dept. of Arctic and Marine Biology, Faculty of Biology, Fisheries and Economics, University of Tromsø, Tromsø, Norway

In this study we aimed to investigate chemodiversity of symbiotic isolates of genus *Nostoc*. Cyanobacteria from *Blasia pusilla*, *Anthoceros punctatus*, *Gunnera tinctoria* and *Gunnera magellanica* from Norway and Chile were isolated and characterized by presence of small peptides in cell-extracts and supernatants. In addition, a range of previously isolated symbiotic strains from other parts of the world and hosts were studied. The characterizations were done by means of MALDI-TOF and ESI-MS. Symbiotic isolates showed great diversity of peptides produced. Even though the majority of peaks represented yet unknown substances we could match a number of peptides with reported secondary metabolites. Among identified peptides we found microcystins, anabaenopeptins, nostopeptolides and microviridins. Our data suggests that symbiotic competence most likely is not restricted by secondary metabolite identity. Moreover a single host plant accepts cyanobionts with different profiles, including strains which are potentially highly toxic.

## P167: Lawsone (2-hydroxy-1,4-naphtoquinone) dependent tellurite biotransformation in anaerobic photosynthetically grown *Rhodobacter capsulatus* cells

**Roberto Borghese, Francesco Francia and Davide Zannoni**

FaBiT – Dept of Pharmacy & BioTechnology, University of Bologna, Bologna, Italy

Bacteria grown on tellurium oxyanions generate variably sized nanoparticles of Te<sup>0</sup> that in a few cases appear as irregular spheres of less than 50 nm in diameter [1]. Unlike bulk material, nanoparticles show peculiar physical, chemical, electronic and biological properties that derive from their dimension. Therefore, the synthesis of monodispersed nanoparticles with different size and shape is a key goal but remains a challenge in nanotechnology due to the use of toxic chemicals on the surface of nanoparticles along with non-polar solvents in the synthesis procedure. Conversely, microbiological methods to generate nanoparticles are regarded as safe, cost-effective and environment-friendly processes although they are at present affected by a series of drawbacks, namely: a) they are time-consuming, b) they lack of a tight control over nanoparticles size distribution, shape and crystal properties, and c) nanoparticles are not monodispersed. On the other hand, recent insights gained from strain isolation, selection, optimization of nanoparticles growth conditions along with the possibility to generate genetically engineered strains overexpressing specific reducing agents, envisage the microbial synthesis of nanoparticles as an interesting field of research [2]. A promising biotechnological application of microbial reduction of metalloids concerns the production of semiconductors nanocrystals known as quantum dots (QDs), having unique electronic and optical properties due to quantum confinement effects. Among various QDs constructed through the use of microorganisms, e.g. ZnS, PdS, CdS and CdSe QDs, cadmium telluride (CdTe) nanocrystals have attracted significant attention for energy, electronics and biomedical applications [2]. Recent studies have shown the utility of redox mediators such as lawsone and anthraquinone-2,6-disulfonate during anaerobic biotransformation of azo dyes, nitroaromatics and polychlorinated compounds, Fe(III) oxides, U(VI), Tc(VII), As(V), and Se(IV) [3, 4]. Here we present a preliminary study on the biotransformation of tellurium oxyanions into Te-nanoprecipitates by photosynthetic cultures of *Rhodobacter capsulatus* as a function of exogenously added lawsone (2-hydroxy-1,4-naphtoquinone). Under these conditions elemental tellurium accumulated outside the cells contrary to the intracellular deposition seen in the absence of added lawsone [5]. External Te-nanoprecipitate can be recovered by filtration or by gradient centrifugation. Rapid flash-spectroscopy and SEM analyses indicated that lawsone strongly interacts with cells not only affecting the electron transfer kinetics but, apparently, also producing cell-shape variation.

[1] Baesman SM et al. Appl Environ Microbiol 2007;73:2135–43. [2] Narayanan KB et al. Adv Colloid Interface Sci 2010;156:1-13. [3] Lu Z et al. Langmuir 2008;24:5445–52. [4] Zhang B et al. Adv Funct Mater 2007;17:486–92. [5] Borghese R et al. Appl Environ Microbiol 2004;70:6595–6602.

## P168: Transformation of the cyanobacterium *Planktothrix* with a synthetic *mcyT* gene encoding a type II thioesterase and its effect on Microcystin production

**Guntram Christiansen and Rainer Kurmayer**

Institut for Limnology, Austrian Academy of Sciences, Mondsee, Austria

Microcystins (MC) are bioactive secondary metabolites produced by several cyanobacterial genera. They are potent inhibitors of eukaryotic protein phosphatases and the biosynthesis of MC is catalyzed by non-ribosomal peptide synthetases (NRPS). Several gene clusters encoding Microcystin synthetases have been isolated from different cyanobacterial species including *Planktothrix agardhii* CYA 126/8. One unique feature of the Microcystin operon from *Planktothrix* is the existence of an associated type II thioesterase (TE II), which is missing in the Microcystin operons isolated from other cyanobacterial genera. Type II thioesterases have been shown to desacetylate miss-primed thiolation domains (T-domains), which have been loaded with an acetylated 4'-phosphopantetein by a dedicated phosphopanteteinyl transferase (PPT). Insertional inactivation of the gene coding for the Microcystin synthetase associated TE II (*mcyT*) in *Planktothrix* resulted in a mutant with a decreased amount of detectable MC (6 ± 1%) compared to the wild type. This shows the critical role of *McyT* for the productivity of the Microcystin synthetase. Quantification of mRNA transcripts of *mcyT* in comparison with a gene encoding for a NRPS enzyme (*mcyB*) of the Microcystin synthetase showed an equimolar mRNA relation. We were interested in the effects of an increased transcription of *mcyT* on the MC productivity in *Planktothrix*. To achieve this we designed a synthetic *mcyT* gene (*mcyTsyn*) under control of two different promoters and terminators. The constructs were introduced into *Planktothrix agardhii* CYA 126/8 and the amounts of MC produced by the mutants carrying the additional *mcyTsyn* gene were determined. First results will be presented.

## P169: Genetic Manipulation of *Anabaena* sp. PCC 7120 Nitrogenase to enhance Photobiological Hydrogen Production

**Hajime Masukawa<sup>1,2</sup>, Kazuhito Inoue<sup>2,3</sup>, Hidehiro Sakurai<sup>2</sup>, Robert P. Hausinger<sup>4,5</sup>**

<sup>1</sup>JST (Japan Science and Technology Agency), PRESTO; <sup>2</sup>Research Institute for Photobiological Hydrogen Production, and <sup>3</sup>Department of Biological Sciences, Kanagawa University, Japan; <sup>4</sup>Department of Microbiology and Molecular Genetics, and <sup>5</sup>Department of Biochemistry and Molecular Biology, Michigan State University, USA

We are proposing large-scale photobiological hydrogen production by mariculture raised cyanobacteria where the microbes convert solar energy into hydrogen with water as the source of electrons to reduce protons (1). The hydrogen gas is produced as the inevitable by-product of nitrogen fixation by nitrogenase, an oxygen-labile enzyme typically containing an iron-molybdenum cofactor (FeMo-co) at the active site. In the absence of N<sub>2</sub>, the enzyme directs all electrons to hydrogen production.

In an effort to increase hydrogen production by this strain, six amino acid residues predicted to reside in the vicinity of the FeMo-co were mutated in an attempt to direct electron flow more selectively toward proton reduction. Many of the 49 variants created were deficient in N<sub>2</sub>-fixing growth and exhibited decreases in their *in vivo* rates of acetylene reduction. Of greater interest, several variants examined under an N<sub>2</sub> atmosphere significantly increased their *in vivo* rates of H<sub>2</sub> production, approximating rates equivalent to those under an Ar atmosphere, and accumulated high levels of H<sub>2</sub> compared to the reference strains. Of the variants tested, the R284H culture exhibited the most dramatically increased levels of accumulated H<sub>2</sub> compared to the reference strain cultures in an atmosphere containing N<sub>2</sub> and O<sub>2</sub> (2) and was able to accumulate H<sub>2</sub> for extended periods over several weeks without changing the culture medium.

1. Sakurai H & Masukawa H (2007) Promoting R & D in photobiological hydrogen production utilizing mariculture-raised cyanobacteria. *Marine Biotechnology* 9(2):128-145; 2. Masukawa H, Inoue K, Sakurai H, Wolk CP, & Hausinger RP (2010) Site-Directed Mutagenesis of the *Anabaena* sp. Strain PCC 7120 Nitrogenase Active Site To Increase Photobiological Hydrogen Production. *Appl Environ Microbiol* 76(20):6741-6750.

## P170: Filamentous N<sub>2</sub>-fixing cyanobacteria - efficient tool for extended H<sub>2</sub> production

**Leino Hannu<sup>1</sup>, Kosourov Sergey<sup>2</sup>, Tsygankov Anatoly<sup>2</sup>, Saari Lyudmila<sup>3</sup>, Sivonen Kaarina<sup>3</sup>, Aro Eva-Mari<sup>1</sup> and Allahverdiyeva Yagut<sup>1</sup>**

<sup>1</sup>Department of Biochemistry and Food Chemistry, Molecular Plant Biology, University of Turku, FI-20014 Turku, Finland; <sup>2</sup>Institute of Basic Biological Problems RAS, Pushchino, Moscow Region, 142290 Russia; <sup>3</sup>Department of Applied Chemistry and Microbiology, University of Helsinki, FI-00014 Helsinki, Finland

We have screened about 400 cyanobacterial strains, isolated from the Baltic Sea and Finnish lakes and maintained at the University of Helsinki Culture Collection, for efficient H<sub>2</sub> producers [1]. Screening revealed several promising cyanobacterial strains producing similar amounts or even more H<sub>2</sub> as the uptake hydrogenase mutant (*ΔhupL*, [2]) of *Anabaena* PCC 7120 specifically engineered to produce higher amounts of H<sub>2</sub>.

One of the best H<sub>2</sub> producers is *Calothrix* 336/3, a filamentous, N<sub>2</sub>-fixing cyanobacterium with ellipsoidal heterocysts located at the base of the filaments. Common feature of *Calothrix* 336/3 is that cells adhere to the substrate. Making use of this important feature of the *Calothrix* 336/3, we applied an immobilization technique to improve H<sub>2</sub> production capacity of this strain. We immobilized the cells within thin Ca<sup>2+</sup>-alginate films [3]. We examined the basic properties of immobilization in Ca<sup>2+</sup>-alginate films in response to the production of H<sub>2</sub> of the *Calothrix* 336/3 strain. We used as a reference strains a model organism *Anabaena* PCC 7120 *ΔhupL*, which allowed us to compare the responses of different strains to alginate entrapment. Immobilization of the *Calothrix* 336/3 cells was most successful and production of H<sub>2</sub> could be measured for a long period after immobilization over several cycles.

In order to compare the capacity of H<sub>2</sub> production by suspension and immobilized cells, the H<sub>2</sub> production assay was performed also in the suspension cultures. The H<sub>2</sub> production reduced significantly after the first cycle in suspension cultures. Despite the fact that the maximum specific rate of H<sub>2</sub> production was comparable between the suspension cultures and alginate-entrapped cells, the entrapped cells produced substantially more H<sub>2</sub> at much longer time course.

H<sub>2</sub> production by both the entrapped cells and suspension cultures was highly dependent on CO<sub>2</sub> level in the headspace. The cells supplemented with 2% and 6% CO<sub>2</sub> demonstrated longer H<sub>2</sub> production capacity over several cycles. The decline in H<sub>2</sub> production by the cells supplemented with CO<sub>2</sub> after several cycles apparently resulted mainly from the N<sub>2</sub> deficiency. This conclusion was supported by the fact that after regular recovery periods on air the cells produced even higher amounts of H<sub>2</sub> during 2 months. Extended H<sub>2</sub> production by the immobilized *Calothrix* 336/3 cells on photobioreactors are in progress.

[1] Allahverdiyeva Y, Leino H, Saari L, Fewer D, Shunmugam S, Sivonen K and Aro EM (2010). *Int J Hyd Energy*. 35(3): 1117-1127; [2] Masukawa H, Mochimaru M & Sakurai H (2002). *App Microbiol Biotech*. 58: 618-624; [3] Leino H, Kosourov S, Saari L, Sivonen K, Tsygankov AA, Aro EM and Allahverdiyeva Y (2012). *Int J Hyd Energy*. 37(1): 151-161

## P171: Genomic analysis and secondary metabolite production in a non-indigenous, toxic cyanobacterium *Cuspidothrix issatschenkoi* (USAČEV) RAJANIEMI ET AL.

**Jan Mareš<sup>1,2</sup>, Pavel Hrouzek<sup>3</sup>, and Eliška Zapomělová<sup>2,4</sup>**

<sup>1</sup>Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic; <sup>2</sup>Department of Botany, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic; <sup>3</sup>Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň, Czech Republic; <sup>4</sup>Institute of Hydrobiology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

In the last thirty years, a planktonic heterocytous cyanobacterium *Cuspidothrix issatschenkoi* (USAČEV) RAJANIEMI ET AL. (= *Aphanizomenon issatschenkoi*) has started spreading from the Caspian region to numerous non-native localities in Europe, North America, Japan, and New Zealand<sup>[1,2]</sup>. It has soon become a frequent component of harmful cyanobacterial blooms producing powerful toxins, especially anatoxin-a<sup>[2,3]</sup>. In our study, we analysed a clonal strain of *C. issatschenkoi* grown from a single filament isolated from a cyanobacterial bloom in fishpond Papež near Dobříš, Czech Republic. The cyanobacterium produced heterocytous neither under natural conditions nor in culture. Thus, it was morphologically hardly separable from *Raphidiopsis mediterranea* SKUJA<sup>[4]</sup>. A high-concentration genomic DNA sample was prepared by pooling multiple displacement amplification products derived from single *Cuspidothrix* filaments, and a draft genome sequence of the strain was generated by shotgun 454 sequencing. A secondary metabolite profile of the strain (focusing on non-ribosomal peptides and other cyanotoxins) was assessed by HPLC-MS and Maldi-TOF techniques. Taxonomic identity of the strain was proved by a robust molecular phylogenetic analysis. The genomic data, including a phylogenomic analysis of the metabolic pathway components of toxin synthesis (especially the non-ribosomal peptide synthetases and polyketide synthetases) was confronted with the actual toxin production of the strain *in vitro*. Our results substantially improve the current knowledge of this potentially harmful cyanobacterial species.

[1] Kaštovský J., Hauer T., Mareš J. et al. (2010) : A review of the alien and expansive species of freshwater cyanobacteria and algae, a case study from the Czech Republic. *Biol. Inv.* 12:3599-3625; [2] Wood S.A., Rasmussen J.P., Holland P.T. et al. 2007: First report of the cyanotoxin anatoxin-a from *Aphanizomenon issatschenkoi* (Cyanobacteria). *J. Phycol.* 43:356-365; [3] Ballot A., Fastner J., Lentz M., and Wiedner C. 2010. First report of anatoxin-a-producing cyanobacterium *Aphanizomenon issatschenkoi* in north-eastern Germany. *Toxicon* 56:964-971; [4] Moustaka-Gouni M., Kormas K.A et al. 2010. Polyphasic evaluation of *Aphanizomenon issatschenkoi* and *Raphidiopsis mediterranea* in a Mediterranean lake. *J. Plankt. Res.* 32:927-936.

## P172: McyL catalyzes the acetylation of microcystins in *Nostoc*

**Julia Österholm<sup>1</sup>, David P. Fewer<sup>1</sup>, Matti Wahlsten<sup>1</sup>, Leo Rouhiainen<sup>1</sup>, Jouni Jokela<sup>1</sup>, Ulla Kaasalainen<sup>2</sup>, Jouko Rikkinen<sup>2</sup>, and Kaarina Sivonen<sup>1</sup>**

<sup>1</sup>Department of Food and Environmental Sciences, Division of Microbiology and <sup>2</sup>Department of Biosciences, University of Helsinki, FI-00014, Helsinki, Finland

Microcystins are a family of cyclic peptide toxins produced by cyanobacteria in freshwater lakes and reservoirs. They are responsible for the toxicosis and death of wild and domestic animals throughout the world. Here we show that the production of acetylated microcystins is catalyzed by an acetyl-CoA dependent acetyltransferase. The occurrence of acetylated and methylated Adda variants in *Nostoc* is the direct consequence of the loss and gain of genes encoding specific tailoring enzymes. The strains containing acetyltransferase gene *mcyL* but lacking methyltransferase gene *mcyJ* produced acetylated microcystins. Phylogenetic analysis demonstrates that McyL belongs to a family of enzymes that inactivate antibiotics through acetylation. We cloned and over-expressed McyL in *E. coli* and showed that the enzyme has a relaxed substrate specificity allowing the enzymatic preparation of semisynthetic propionated or butyrylated derivatives of microcystin. This study sheds light on the evolutionary origins and genetic diversity of an important class of enzymes involved in antibiotic resistance.

## P173: Exopolysaccharide of freshwater unicellular cyanobacterium *Cyanothece* sp. Vietnam 01; synthesis and physicochemical characterization

**Kaori Ohki<sup>1</sup>, Nguyen Quyen Thi Le<sup>2</sup>, Shinya Yoshikawa<sup>1</sup>, Mitsunobu Kamiya<sup>1</sup>, Maiko Okajima<sup>2</sup>, Tatsuo Kaneko<sup>2</sup> and Tran Hang Thi<sup>3</sup>**

<sup>1</sup>Department of Marine Bioscience, Fukui Prefectural University, Obama, Fukui, Japan; <sup>2</sup>School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Ishikawa, Japan; <sup>3</sup>Faculty of Technology of Chemistry, College of Chemistry, Ministry of Industry and Trade, Tien Kien, Lam Thao, Phu Tho, Viet Nam

Unicellular cyanobacterium which produces a large amount of exopolysaccharide (EPS) has been isolated from the rice field in Phu Tho area, Vietnam. Cells of isolate were rod-shaped with 8 to 10  $\mu\text{m}$  in diameter, lacked structured sheaths and divided by transverse binary fission in a single plain. Cells were surrounded by gelatinous materials, and formed large aggregate in a culture medium. The major photosynthetic pigments were chlorophyll *a*, C-type phycocyanin and phycoerythrin. Phylogenetical analysis using the partial sequence of 16S rRNA gene revealed that this isolate was closely related to *Cyanothece* sp. PCC. 7822. The isolate, named *Cyanothece* sp. Vietnam 01, was grown in a liquid medium containing salts (1.2 g NaCl, 0.55 g  $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.2 g  $\text{Na}_2\text{SO}_4$ , 68 mg  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 5 mg KBr, 0.15 mg NaF, 1.5 mg  $\text{H}_3\text{BO}_3$ , and 0.85 mg  $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$   $\text{L}^{-1}$ ) and nutrients (0.5 g  $\text{NaNO}_3$ , 0.05 g  $\text{K}_2\text{HPO}_4$ , 0.4 g  $\text{NaHCO}_3$ , 1 ml of metal and vitamin solutions [1]) under 12 h light / 12 h dark cycles. Cells were able to grow under the wide range of temperature (25 to 40°C), whereas it died lower than 20°C. The isolate had an ability of nitrogen fixation, and grew in the medium without combined nitrogen. The maximum growth rates (increase in chlorophyll *a* concentration) at 30°C under the light intensity of 40  $\mu\text{mol m}^{-2} \cdot \text{s}^{-1}$  were about 0.7 and 0.3  $\text{d}^{-1}$  in the medium with and without combined nitrogen, respectively. The EPS contents on dry weight base altered from 30 to 80% depending on temperature and/or light intensity. Cells in stationary growth phase were collected onto a plastic basket and kept at -30°C until extraction of the EPS.

The EPS was extracted using NaOH aqueous solution with agitation and heating, and was obtained as white fibrous materials by an alcohol precipitation method [2]. The absolute molecular weight of the EPS was estimated as  $4.5 \times 10^7$ -g  $\text{mol}^{-1}$  (by multi-angle static light scattering). A FT-IR spectrum of the EPS showed several distinct peaks at wavenumbers of 1040 (C-OH stretch), 1260  $\text{cm}^{-1}$  ( $\text{SO}_2$  asymmetrical stretch), 1400 ( $\text{CH}_2$  wagging), 1620 (C=O stretch), 2800 (C-H stretch), and 3400 (O-H stretch). The pattern of FT-MS spectrum indicated that the EPS had carbonyl and sulfate groups, as well as the typical functional groups of sugars. From the results of the carbazole-sulfuric acid method (525 nm), the uronic acid content was estimated at 23 mol%. The ratio of S was estimated to 1.66 wt% per total monosaccharide from the results of elemental analysis (CHN S). A chromatography/mass spectroscopy (GC/MS) of trimethylsilylated samples of methanolized EPS indicated that the constituent monosaccharides were Rha, Glc, Gal, Xyl, Man, Fuc, Ara, Rib and unknown sugars (Unk) with a composition of 38.6: 13.8: 4.8: 4.8: 2.4: 3.5 : 2.0 : 0.6 and 6.5.

[1] Ohki et al. Mar. Biol. 91: 9-13 (1986); [2] Okajima-Kaneko et al. Pure Appl. Chem. 79: 2039-2046 (2007)

## P174: GENOMIC INSIGHTS INTO SECONDARY METABOLISM OF THE NATURAL PRODUCT-RICH CYANOBACTERIUM *MOOREA BOUILLONII*

**Emily A. Monroe<sup>1</sup>, Valery Lesin<sup>2</sup>, Alexander Sirotkin<sup>2</sup>, Mikhail Dvorkin<sup>2</sup>, Pavel Pevzner<sup>2,3</sup>, William H. Gerwick<sup>1,4</sup> and Lena Gerwick<sup>1</sup>**

<sup>1</sup> Scripps Institution of Oceanography, University of California San Diego, USA; <sup>2</sup> St. Petersburg Academic University of the Russian Academy of Sciences, Russia; <sup>3</sup> Department of Computer Science, University of California San Diego, USA; <sup>4</sup> Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, USA

Marine cyanobacteria have become an important source for bioactive and structurally diverse natural products. *Moorea bouillonii* (formerly *Lyngbya bouillonii*) is known to produce about 30 different natural products; however, the true natural product potential and life strategies of *Moorea* strains remain poorly investigated. To further explore the natural product potential of a *M. bouillonii* strain and gain insights into other life history characteristics, we sequenced the genome of the type-strain, *M. bouillonii* PNG5-198, using paired-end Illumina sequencing. The 8.6 Mb genome was assembled using the newly developed SPAdes algorithm followed by error correction using the Bayes Hammer program. The genome is estimated to contain 7241 predicted protein coding genes, which is consistent with the genome size and protein coding genes of another recently sequenced *Moorea* strain, *M. producin* 3L<sup>1</sup>. In addition to contigs consistent with the published biosynthetic pathway for the anti-cancer compound apratoxin A<sup>2</sup>, there is also a pathway consistent with lyngbyabellin A biosynthesis, another cytotoxic metabolite from this species. The biosynthetic pathway for lyngbyabellin A has significant similarity to the hectochlorin biosynthetic pathway, a structurally similar bioactive compound from a closely-related species, *M. producin*, which raises interesting questions regarding the evolution of secondary metabolite pathways in marine cyanobacteria. Five additional contigs in the genome contain sequences relevant to secondary metabolism and may reveal the biosynthetic capacity for novel compounds not yet identified from this strain. Genome sequencing has provided insight into secondary metabolism capacity and evolution and continues to be a useful tool in aiding in natural product discovery from marine cyanobacteria. This project was funded from grants: NIH Grant 2R01 CA10887406 to W.H.G and L.G, and Russian megagrant to P.A.P.

<sup>1</sup> Jones AC, Monroe EA, Podell S, Klages S, Hess W, Esquenazi E, Kube M, Niessen S, Hoover H, Rothmann M, Reinhardt R, Lasken R, Yates 3rd JR, Burkart M, Allen E Gerwick WH, Gerwick L. Secondary metabolism, complex gene regulation, and nitrogen requirements of the marine cyanobacterium *Lyngbya majuscula* 3L are revealed through genome sequencing and proteomic analyses. PNAS, 2011 May 24;108(21):8815-20; <sup>2</sup> Grindberg RV, Ishoey T, Brinza D, Esquenazi E, Coates RC, Gerwick L, Dorrestein P, Pevzner P, Lasken R, Gerwick WH. Single cell genome amplification to accelerate natural product pathway isolation from elusive microorganisms. PLoS One, 2011 Apr 12;6(4):e18565



**P175: In vitro inhibitory activity of extracts of Brazilian Cyanobacteria against Herpes simplex viruses****Luciana R. Carvalho<sup>1</sup>, Rebeca M. Oliveira<sup>2</sup>, Gabriela S. Mendes<sup>2</sup>, Geanne A. Conserva<sup>1</sup>, Célia L. Sant' Anna<sup>1</sup>, and Maria Teresa V. Romanos<sup>2</sup>**<sup>1</sup>Núcleo de Pesquisa em Ficologia, Instituto de Botânica, São Paulo, Brazil; <sup>2</sup>Laboratório Experimental de Drogas Antivirais e Citotóxicas, Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Herpes simplex viruses (HSV) infections are among the most common diseases throughout the world, and it has been estimated that 60 - 95% of the adult population are infected with them. Generally, HSV-1 has been associated with oro-labial disease, with most infections occurring during childhood, and HSV-2, with genital infections acquired during sexual contacts. The incidence and severity of HSV-related pathologies have increased recently, mainly among patients with reduced cellular immunity, as in bone marrow transplant recipients and in bearers of acquired immunodeficiency syndrome (AIDS). These patients are treated with long-term antiviral therapy, which can lead to the selection of resistant variants. Thus, new anti-HSV drugs are urgently needed. Cyanobacteria are prolific producers of highly bioactive compounds, some of them displaying interesting antiviral activities[1]. Cyanovirin-N is a protein synthesized by *Nostoc ellipsoforum*, which, besides inhibiting HIV and influenza virus, it blocks HSV-1 entry into cells and prevents membrane fusion mediated by HSV glycoproteins; three *Microcystis* species showed remarkable activity against influenza virus and, estuarine cyanobacterial extracts are active against HSV-1[2]. In this work, acetic acid 0.1 M extracts of the cyanobacterial species *Lingbya* sp. CCIBt 862, *Phormidium* sp. CCIBt 1018, *Nostoc* sp. CCIBt 1028 and *Geitlerinema unigranulatum* CCIBt 971 were evaluated against HSV-1 and HSV-2. Antiviral assays were performed on Vero cell cultures, in the presence of the cyanobacteria extracts at non-cytotoxic concentrations. *Lingbya* sp. extract showed potent inhibitory activity against HSV-1 (94.4%) with  $ED_{50} = 108 \mu\text{g/mL}$  and no action against HSV-2. With regard to activity against HSV-1, *Phormidium* sp. caused 96% inhibition with  $ED_{50} = 383 \mu\text{g/mL}$ ; *Nostoc* sp., 94.4% with  $ED_{50} = 39 \mu\text{g/mL}$ , and *G. unigranulatum*, 60.2% with  $ED_{50} = 57 \mu\text{g/mL}$ . In relation to HSV-2, *Phormidium* sp. showed 99.7% inhibition, with  $ED_{50} = 43 \mu\text{g/mL}$ ; *Nostoc* sp. 96.4% with  $ED_{50} = 48 \mu\text{g/mL}$  and *G. unigranulatum*, 87.9% with  $ED_{50} = 49 \mu\text{g/mL}$ . Our results are in agreement with the ones displayed in literature about the cyanobacterial extracts (compounds) inhibitory activity against HSV and indicate that three of the four studied cyanobacterial extracts have potential to provide novel drug candidates. Bioactive compounds identification studies will be performed in order to elucidate their structures and mechanism of action.

[1].Sheng Xiong *et al.*, 2010. Appl. Microbiol. Biotechnol. 86(3): 805-812; [2].Viviana R. Lopes *et al.*, 2011. Toxicol In Vitro 25(4):944-950**P176: Citotoxicity of marine cyanobacteria from the portuguese coast against breast cancer cells****Margarida Costa<sup>1</sup>; Pedro Leão<sup>1</sup>; Maria João Ribeiro<sup>1,2,3</sup>; João Costa-Rodrigues<sup>4</sup>; Mónica Garcia<sup>4</sup>; Piedade Barros<sup>3</sup>, Helena Fernandes<sup>4</sup>; Vítor Vasconcelos<sup>1,5</sup>; Rosário Martins<sup>1,3</sup>**<sup>1</sup>CIIMAR, University of Porto, 4050-123 Porto, Portugal; <sup>2</sup>Superior School of Health Technology of Porto, Polytechnic Institute of Porto, 4400-330 Vila Nova de Gaia, Portugal; <sup>3</sup>University of Aveiro, 3810-193 Aveiro, Portugal; <sup>4</sup>Faculty of Dental Medicine, University of Porto, 4200-393 Porto, Portugal; <sup>5</sup>Faculty of Sciences, University of Porto 4169-007 Porto, Portugal

The marine environment possesses an unmeasured chemical and biological diversity, being considered an extraordinary resource for the discovery of new therapeutic drugs. Between others, marine cyanobacteria have emerged as a prominent source of bioactive secondary metabolites with a promising anticancer potential, as the described and intensively studied dolastatins. These compounds have been mainly isolated from the genera *Lingbya*, *Microcoleus* and *Symploca*, which grow at high densities in tropical and subtropical regions. Others, such as *Cyanobium*, *Synechocystis*, *Synechococcus*, *Leptolyngbya* and *Pseudoanabaena*, contribute only with a few described natural products. These last genera represent a large fraction of the marine cyanobacteria culture collection, maintained in the Laboratory of Ecotoxicology Genomics and Evolution at CIIMAR, Porto, Portugal. In order to evaluate the breast anticancer potential of strains belonging to these unexplored genera, we have been performing a cytotoxicity screening using human breast cancer cells. The assays are performed using a crude extract, obtained with a dichloromethane: methanol extraction (2:1), and three more extracts, obtained by a crude extract fractionation in a Si column chromatography. The cytotoxicity is evaluated by a MTT assay at 24, 48 and 72 hours and confirmed using a LDH assay. The results show that, inside of a group of 28 selected cyanobacteria strains, 5 can be detached as producers of cancer cells growth inhibitors. The present study has allowed us to shortlist strains for chemical exploration of their anticancer constituents.



## P177: Cytotoxic activity of marine cyanobacteria against cancer cell lines

**Maria João Ribeiro<sup>1,2\*</sup>, Margarida Costa<sup>1</sup>, Pedro Leão<sup>1</sup>, Piedade Barros<sup>3</sup>, Vitor Vasconcelos<sup>1,4</sup>, Rosário Martins<sup>1,3,5</sup>**

<sup>1</sup>CIIMAR/CIMAR, Center for Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal; <sup>2</sup>Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; <sup>3</sup>Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, 4400-330 Vila Nova de Gaia, Portugal; <sup>4</sup>Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal; <sup>5</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal

Marine cyanobacteria possess an extensive capacity to produce compounds that were found to induce biological activities against cell lines and organisms [1]. Many of the marine cyanobacteria compounds already described were found to induce toxicity against cancer cells [2]. Nearly all of these compounds derive from strains of the genera *Lyngbya* and *Leptolyngbya*. Nevertheless, there are other genera that have been largely overlooked, mainly because, under natural conditions they occur at low densities. Here we present the results of a screening work that aimed to evaluate the anti-cancer potential of five cyanobacterial strains from the genera *Cyanobium*, *Leptolyngbya*, *Romeria* and *Synechocystis*, isolated from the Portuguese coast. Crude extracts obtained by dichloromethane and methanol extractions and three fractions obtained using Si column chromatography with a gradient from 100% hexane, to 100% ethyl acetate to 100% methanol were tested for cytotoxicity against the human cancer cell lines colon adenocarcinoma (HT29 and RKO), hepatocellular carcinoma (HepG2), neuroblastoma (SH-SY5Y) and osteosarcoma (MG63). (Growth inhibition/proliferation was evaluated by the MTT assay at 24, 48 and 72 hours. The results show controversial effects of the cyanobacteria extracts, since both inhibitory and stimulatory effects on cell growth within the same strain were observed.

[1] Nagarajan, M., Maruthanayagam, V., and Sundararaman, M. (2012). A review of pharmacological and toxicological potentials of marine cyanobacterial metabolites. *J Appl Toxicol* 32, 153-185; [2] Sithranga Boopathy, N., and Kathiresan, K. (2010). Anticancer drugs from marine flora: an overview. *J Oncol* 2010, 214186.  
\*mrm@estsp.ipp.pt

## P178: The response of different human and murine cell lines to crude extracts and pure chemical constituents of cyanobacteria

**Pavel Hrouzek<sup>1,2</sup>, Alexandra Kapuscik<sup>1,2</sup>, Jan Vacek<sup>3</sup>, Pavel Kosina<sup>3</sup>, Martina Zatloukalová<sup>3</sup>, Kateřina Voráčková<sup>1,2</sup>, Petr Tomek<sup>1</sup>, Jindra Pajchlová<sup>1,2</sup>, Jitka Ulrichová<sup>3</sup> and Jiří Kopecký<sup>1,2</sup>**

<sup>1</sup> Institute of Microbiology, Department of Phototrophic microorganisms – ALGATECH, Academy of Sciences of the Czech Republic, Opatovický Mlýn, 379 81 Třeboň, Czech Republic; <sup>2</sup> Faculty of Sciences, University of South Bohemia, Branišovská 31, 370 05 České Budějovice, Czech Republic; <sup>3</sup> Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacký University in Olomouc, Hněvotínská 3, 775 15 Olomouc, Czech Republic

The toxicity of cyanobacteria and the occurrence of the cyanobacterial water blooms is permanent problem in water management, agriculture and in recreation areas in most of the countries. The ability of cyanobacteria to produce broad spectrum of secondary metabolites, including substances toxic to human, is well known for last three decades. Beside traditionally monitored hepatotoxins and neurotoxins, there exists a large group of cyanobacterial compounds that target basic mechanisms required for a single cell survival. Such compounds are usually grouped into an artificial class of cytotoxins. Up till now around 80 cyanobacterial cytotoxins have been known. Most of them are general cytotoxins which can affect human health, moreover, majority of cyanobacterial compounds and their effect on human remains unknown. Thus methodology for determination of toxicity (cytotoxicity) of cyanobacterial metabolites and their mixtures is needed. In order to test cytotoxicity of cyanobacterial compounds, the selected cyanobacterial extracts have been tested for the cytotoxicity to four cell lines of human (HeLa and HepG2) and murine (YAC-1, Sp2) origin. These data were compared with the standard cytotoxicity assay performed on murine fibroblasts (BALB/c). Finally a subset of the extracts was tested for its toxicity to the cultured hepatocytes as one of the *in vitro* model in human toxicological studies. Roughly one third of cyanobacterial extracts has been found to cause cytotoxicity to the cell lines *in vitro*. Although higher sensitivity to crude cyanobacterial extracts has been found in some cell lines (mainly for murine lymphoid cell lines YAC-1 and Sp2). The strong correlation in cytotoxic effects (over 70%,  $p=0.0001$ ) has been found among the effects caused by cyanobacterial extracts to the cell lines of HeLa, HepG2 and the standard assay using BALB/c. This result suggests that most of cyanobacterial metabolites can be generally cytotoxic with low organ targeted specificity. In present contribution we discuss possible development of standard method for cytotoxicity testing and estimation of possible threat of cyanobacterial metabolites to human. The isolation of new cytotoxins (puwainaphycin F and muscotoxin) is presented and their cytotoxicity in pure state and in crude extract is compared.

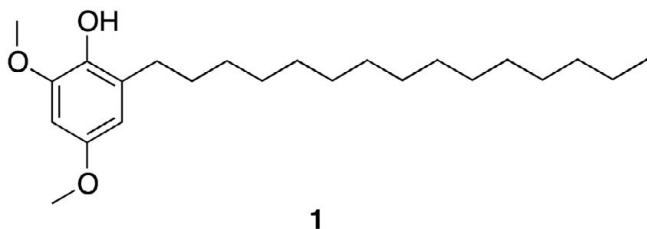
## P179: Isolation of Hierridin B from a culturable *Cyanobium* sp. strain isolated from the Portuguese coast

Pedro N. Leão<sup>1,2,\*</sup>, Alban R. Pereira<sup>2</sup>, Margarida Costa<sup>1</sup>, Vitor Ramos<sup>1</sup>, Rosário Martins<sup>1,3,4</sup>, William H. Gerwick<sup>2,5</sup> and Vitor Vasconcelos<sup>1,6</sup>

<sup>1</sup>CIIMAR/CIMAR, Center for Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal; <sup>2</sup>Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA, 92093-0636, USA; <sup>3</sup>Escola Superior de Tecnologia da Saúde do Porto, Instituto Politécnico do Porto, 4400-330 Vila Nova de Gaia, Portugal; <sup>4</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal; <sup>5</sup>Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, 92093-0636, USA; <sup>6</sup>Department of Biology, Faculty of Sciences, University of Porto, Rua do Campo Alegre, 4169-007 Porto, Portugal

Cyanobacteria are a genetically diverse group of phototrophic prokaryotes. On a secondary metabolite perspective, however, filamentous and benthic forms have been the major sources of compounds isolated from these organisms. Nevertheless, unicellular, free-living, planktonic cyanobacteria also contain enzymatic machinery for secondary metabolite biosynthesis. Here, we report the production of hierridin B (**1**) by the planktonic *Cyanobium* sp. strain LEGE 06113. This strain was isolated from an intertidal sample collected at Aguda beach, northern Portugal.

Isolation of pure hierridin B was achieved by NMR-guided investigation of a non-polar vacuum liquid chromatography (VLC) fraction. The agreement of <sup>1</sup>H NMR chemical shifts and coupling constants with the literature values for hierridin B isolated from *Phormidium ectocarpi* [1] formed the basis for dereplication. This metabolite features a 2,4,6-substituted phenol moiety with two methoxy groups and one long aliphatic chain as the substituents. Hierridin B has been previously shown to have antiplasmodial activity. Here we present the results of investigations of its bioactivity profile in other systems. In addition, *Cyanobium* genome data available in the databases was mined for genes that may be involved in the biosynthesis of hierridin B. As a fast-growing, unicellular strain, culturing of *Cyanobium* sp. LEGE 06113 presents as a convenient system to obtain this compound in relatively high amounts and further explore its activity profile.



[1] Papendorf O, König GM, Wright AD (1998) *Phytochemistry* 49(8):2383-2386.

## P180: Genotype determined microcystin content in *Planktothrix* spp.

Rainer Kurmayer, and Guntram Christiansen

Institute for Limnology, Austrian Academy of Sciences, Mondsee, Austria e-mail: rainer.kurmayer@oeaw.ac.at

Microcystins (MC) are bioactive secondary metabolites produced by several cyanobacterial genera. Although MC synthesis of a specific MC-producing isolate is continuous and cannot be induced by environmental conditions it is known that individual, MC-producing isolates differ in their MC content by an order of magnitude, e.g. from 0.5–5 microgram of MC per mg dry weight [1]. The biosynthesis of MC is catalyzed by non-ribosomal peptide synthesis and several *mcy* gene clusters encoding MC synthetases have been isolated from different cyanobacterial species. Our aim is to identify factors leading to the observed differences in MC content within forty MC-producing isolates of *Planktothrix agardhii* and *P. rubescens*.

The *mcy* gene cluster of *Planktothrix* is consisting of nine genes transcribed in a bi-directional operon [2]. The majority of the intergenic spacer regions was found invariable. Only within the largest spacer between the *mcyE* and the *mcyG* gene (274 bp) significant genetic variation due to the insertion of transposable elements (1433 bp), deletions (128 bp) was found. Further within the bi-directional promoter region (*mcyTD*), the insertion of a 1240-bp region coding for two hypothetical proteins was observed.

In relation to two reference genes (phycocyanin, RNA polymerase) a relationship between the transcript amount of individual genes of the *mcy* gene cluster and the MC content among all *Planktothrix* strains was found. The transcripts of the genes *mcyG*, H, A located downstream of the intergenic spacer region *mcyEG* were found as strongest predictor variables. Notably, the strains carrying a 128bp deletion within *mcyEG* showed a high MC content and a weaker relationship between the transcript amount and MC content.

These results show for the first time that individual *mcy* genotypes differ significantly in the *mcy* transcript amount which can explain a significant part of the variation of the MC content among isolates.

[1] Kosol, S., Schmidt, J., and Kurmayer, R. (2009): Variation in peptide net production and growth among strains of the toxic cyanobacterium *Planktothrix* spp. *Eur. J. Phycol.* 44:49-62; [2] Christiansen, G., Fastner, J., Erhard, M., Borner, T., and Dittmann, E. (2003) Microcystin biosynthesis in *Planktothrix*: Genes, evolution, and manipulation. *J. Bacteriol.* 185:564-572.

## P181: $\alpha$ -Carotene Found Only in Unusual Chlorophyll-Containing Cyanobacteria, *Acaryochloris* and *Prochlorococcus*, among Prokaryotes

**Shinichi Takaichi<sup>1</sup>, Hiroko Uchida<sup>2</sup>, Akio Murakami<sup>2</sup>, Euichi Hirose<sup>3</sup>, Mari Mochimaru<sup>4</sup>, Tohru Tsuchiya<sup>5</sup>, and Mamoru Mimuro<sup>5</sup>**

<sup>1</sup>Department of Biology, Nippon Medical School, Nakahara, Kawasaki, 211-0063 Japan; <sup>2</sup>Kobe University of Research Center of Inland Seas, Awaji, 656-2401 Japan; <sup>3</sup>Faculty of Science, University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan; <sup>4</sup>Department of Natural Sciences, Komazawa University, Setagaya, Tokyo, 154-8525 Japan; <sup>5</sup>Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, 606-8501 Japan

Among all prokaryotes, only two cyanobacterial genera, *Acaryochloris* and *Prochlorococcus*, have been reported exceptionally to have  $\alpha$ -carotene, which is found among many phototrophic eukaryotes [1]. We reexamined carefully identification of the carotenoids including chirality of the unusual cyanobacteria containing diversified Chls, and analyzed the carotenoid compositions. On one hand, Chl *d*-dominating *Acaryochloris* (two strains) and divinyl-Chl *a* and divinyl-Chl *b*-containing *Prochlorococcus* (three strains) had both  $\alpha$ -carotene and minor  $\beta$ -carotene, and zeaxanthin, while Chl *b*-containing *Prochlorothrix* (one strain) and *Prochloron* (three isolates) had only  $\beta$ -carotene and zeaxanthin. Thus, the capability to synthesize  $\alpha$ -carotene seemed to be acquired in both *Acaryochloris* and *Prochlorococcus*, and further we found that  $\alpha$ -carotene in both cyanobacteria had the opposite chirality at C-6'; exceptional (6'S)-type in *Acaryochloris* and usual (6'R)-type in *Prochlorococcus* as reported in some eukaryotic algae and land plants. The results were the first evidence for natural occurrence and biosynthesis of exceptional (6'S)- $\alpha$ -carotene. All of zeaxanthin in these species were usual (3R,3'R)-type. Based on the identification of the carotenoids and on the genome sequence data in these cyanobacteria, we proposed a biosynthetic pathway of the carotenoids and their participating genes and enzymes.

[1] S. Takaichi (2011) Carotenoids in algae: distributions, biosynthesis and functions. Mar. Drugs 9: 1101-1118.

## P182: The production of the odorous metabolites geosmin and 2-methylisoborneol in cyanobacteria

**Suvi Känä, Gonzalo V. Gomez-Saez, Anne Rantala-Ylinen, Jouni Jokela, David P. Fewer and Kaarina Sivonen**

Department of Food and Environmental Sciences, Division of Microbiology, University of Helsinki, Finland

Geosmin and 2-methylisoborneol (2-MIB) are odorous metabolites produced by some cyanobacteria and actinobacteria. These secondary metabolites cause earthy and/or musty odour and taste for example in freshwater, drinking water or fish. Cyanobacteria are the major producers of geosmin and 2-MIB in water. Humans have a very low odor threshold for these metabolites. The biosynthetic genes of geosmin and 2-MIB have been recently discovered from cyanobacteria. In this study, we screened 100 cyanobacterial strains from 20 different genera in order to identify possible producers of these odorous metabolites. Identification was made with solid-phase microextraction coupled with gas-chromatography/mass-spectrometry (SPME GC-MS). The geosmin and 2-MIB biosynthetic genes were also studied using a PCR. Cyanobacteria specific primers were designed to detect part of the *geoA1* gene involved in synthesis of geosmin and 2-MIB synthase gene. In this study, 21 geosmin producers were found with SPME GC-MS from six different genera: *Nostoc*, *Aphanizomenon*, *Oscillatoria*, *Planktothrix*, *Cylindrospermum* and *Calothrix*. Two 2-MIB producers were found belonging to genera *Oscillatoria* and *Planktothrix*. Results of the PCR were consistent with chemical detection. Amplified geosmin and 2-MIB genes were sequenced in order to find out conserved gene regions for further optimization of primers used for detection of the potential producers. Studies will be complemented by phylogenetic analysis of the different cyanobacterial producer strains compared to *Streptomyces* producers of geosmin and 2-MIB.

## P183: Diversity and occurrence of the potent membrane damaging anabaenolysins in cyanobacteria

**Tânia Keiko Shishido, Clara-Theresia Kumer, Jouni Jokela, David P. Fewer, Matti Wahlsten and Kaarina Sivonen \***

Department of Food and Environmental Sciences, Division of Microbiology, University of Helsinki, P.O. Box 56, Viikki Biocenter (Viikinkaari 9), FIN-00014, Helsinki, Finland

Cyanobacteria synthesize a wide range of non-ribosomal peptides with bioactivity against other organisms. Microcystin, nodularin, cylindrospermopsin and saxitoxin are the most commonly detected toxins. Anabaenolysins are new cytotoxins which were identified from *Anabaena* strains isolated from the Baltic Sea (Jokela *et al.*). These are low molecular weight cyclic lipopeptides which contain a fatty acid chain and two non-proteinogenic amino acids and are most likely produced by a non-ribosomal pathway. These potent cytotoxins induce rapid lysis of several human cell lines and hemolysis of red blood cells (Jokela *et al.*). Here we analyze the occurrence of these new cytotoxins in different cyanobacteria and examine their bioactivity. Nine strains belonging to genera *Anabaena* were shown to produce anabaenolysins A and B. These strains were isolated from benthic, epiphytic, epilithic and sediment habitats from the Baltic Sea. Anabaenolysin C, a new variant, was detected in *Anabaena* sp. XPORK13A extract. Anabaenolysin C was found to be mostly intracellular in a growth experiment. Bioactivity tests against bacteria, algae and zooplankton gave negative results but anabaenolysins inhibited the growth of cyanobacteria, mold and yeast. Interestingly, a synergistic effect between anabaenolysin and a hydrophilic compound (s) in the cyanobacterial extract was found. Phylogenetic analysis with 16S rRNA gene placed most of the isolates producing anabaenolysins together with benthic *Anabaena*. The draft genome of the strain XSPORK2A (under sequencing) will reveal the biosynthetic gene cluster responsible for anabaenolysin biosynthesis and thereafter more PCR screenings become possible. We will also identify the compound causing the synergistic effect. Anabaenolysins produced by benthic cyanobacteria may have a possible ecological role in the protection against pathogenic fungi and confer an advantage during competition with other cyanobacteria.

Jokela J, Oftedal L, Herfindal L, Permi P, Wahlsten M, Døskeland S O and Sivonen K. Anabaenolysins, novel cytolytic lipopeptides from benthic *Anabaena* cyanobacteria. (manuscript in review).

## P184: Combined high resolution microscopy techniques for determining the *in vivo* effect and sequestration capacity of lead in phototrophic bacteria

**Juan Maldonado, Elia Diestra, Mireia Burnat, Antonio Solé and Isabel Esteve**

Department de Genètica i Microbiologia, Universitat Autònoma de Barcelona, Edifici C, Campus de la UAB, Bellaterra (Cerdanyola del Vallès), 08193 Barcelona, Spain

Microbial mats are laminated benthic ecosystems made up of microorganisms having diversely coloured layers due to the photo-synthetic pigments of cyanobacteria, algae and purple anoxygenic phototrophic bacteria.

For many years now, our group of work has been studying the microbial mats in the Ebro Delta (Tarragona, Spain). The Ebro Delta is a protected area but it is currently subjected to anthropogenic pollution by heavy metals, making the microorganisms living in these ecosystems a subject of great interest when analyzing their ability to sequester metals.

Our group has optimized different high-resolution microscopy techniques such as Confocal laser scanning microscopy (CLSM), Transmission electron microscopy (TEM) and Scanning electron microscopy (SEM), these last two techniques coupled to an Energy dispersive X-ray detector (EDX). The CLSM coupled to a spectrofluorometric detector (CLSM-*lscan*) was applied in determining the *in vivo* effect of lead (Pb) in phototrophic microorganisms (tolerance/resistance), and the CLSM and image analysis (CLSM-IA) were used in determining changes in total and individual cyanobacteria biomass. Additionally, the electron microscopic techniques were utilized in determining the ability of these microorganisms to capture metal both externally in extrapolymeric substances (EPS) and internally, in polyphosphate inclusions (PP).

For this purpose, we used different cyanobacteria from Pasteur culture collection (*Oscillatoria* sp. PCC 7515, *Chroococcus* sp. PCC 9106 and *Spirulina* sp. PCC 6313) and *Microcoleus* sp. and the microalga DE2009 both isolated from Ebro Delta microbial mats. Pb was selected because the Ebro River is polluted by this metal and also because it is a non-essential toxic metal.

An inverse correlation between the mean fluorescence intensity and the concentration of the metal used have been demonstrated in all phototrophic microorganisms tested CLSM-*lscan*. On the other hand, the SEM-EDX and TEM-EDX analysis shows that all phototrophic microorganisms have the ability to accumulate Pb in EPS and in PP inclusions [1], [2].

Experiments made in unpolluted and polluted microcosms, demonstrate that cyanobacteria from the polluted microcosm accumulate Pb in PP inclusions, whilst no Pb was detected in the unpolluted microcosm by means of TEM-EDX.

Finally, the TEM-EDX analyses spectra from PP inclusions of different cyanobacteria from Ebro Delta microbial mats samples, demonstrate that no type of metal pollution was detected. It can be deduced that this ecosystem was pristine during the sampling procedure [3].

In conclusion, the combination of the techniques outlined here provides valuable information to select cyanobacteria as bioindicators of metal pollution and its potential for bioremediation.

[1] Maldonado et al. 2010 J Hazard Mater 183: 44–50; [2] Maldonado et al. 2011 Aquat Toxicol 104: 135–144; [3] Esteve et al. 2012 (Sent as a chapter book In: Cyanobacteria: Toxicity, Ecology and Management)

## P185: Screening for PKS and NRPS genes in cyanobacteria isolated from the intertidal zones of the Portuguese coast

**Ângela Brito<sup>1,2</sup>, Joana Gaifem<sup>1</sup>, Marta Vaz Mendes<sup>1</sup>, Vitor Vasconcelos<sup>2,3</sup> and Paula Tamagnini<sup>1,2</sup>**

<sup>1</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; <sup>2</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; <sup>3</sup>CIMAR/CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Porto, Portugal

Cyanobacteria are important primary producers and many are able to fix atmospheric nitrogen, playing a key role in the marine environment. In addition to their ecological importance, they are also recognized as a prolific source of biologically active natural products, some of which are toxic to a wide array of organisms. Continental Portugal has an extensive coastline facing the North Atlantic Ocean. It is one of the warmest European countries, and its near-shore wave energy has a strong spatial and seasonal variability, resulting in a rather complex and highly dynamic set of intertidal ecosystems. However, not much is known about the diversity of cyanobacteria along these zones. Previously, cyanobacterial strains were isolated from 9 sites on the Portuguese South and West coasts, cultured and deposited at LEGE Culture Collection (CIIMAR, Porto, Portugal). These organisms were characterized morphologically (light and electron microscopy) and by a molecular approach (partial 16S rRNA, *nifH*, *nifK*, *mcyA*, *mcyE/ndaF* and *sxtI* genes). The morphological analyses revealed 35 morphotypes (15 genera and 16 species) belonging to 4 cyanobacterial Orders/Sub-sections. The dominant group among the isolates were the Oscillatoriales. There is a broad congruence between morphological and molecular assignments. The 16S rRNA gene sequences of 9 strains had less than 97% similarity compared to the sequences in the databases, revealing novel cyanobacterial diversity. One-third of the isolates are potential N<sub>2</sub>-fixers, as they exhibit heterocysts or the presence of *nif* genes was demonstrated by PCR. Additionally, no conventional freshwater toxins genes were detected by PCR screening [1]. Nevertheless, Martins *et al.* [2] demonstrated that extracts of *Synechocystis* and *Synechococcus* strains were toxic to marine invertebrates and it is well-known that cyanobacteria produce a variety of biological active products [3]. Concerning their structure, the majority of these metabolites are polyketides (PK), non-ribosomal peptides (NRP) or a hybrid of the two. The aim of this work is to evaluate the potential of the isolated strains to produce novel bioactive compounds. For this purpose, the organisms were screened for the presence of genes encoding non-ribosomal peptide synthetase (NRPS) and polyketide synthase (PKS) by PCR. Positive results were obtained for more than 80% of the strains tested. The sequences obtained are being used to predict the putative metabolites, using an *in silico* approach. These results will provide a useful insight for future metabolomic studies.

[1] Brito et al (2012). *Syst. Appl. Microbiol.* 35: 110-119; [2] Martins et al (2007) *Toxicon.* 50: 791-799; [3] Jones et al (2010). *Nat. Prod. Rep.* 27: 1048-1065

## P186: Simultaneous detection of cylindrospermopsin and saxitoxin synthetase genes in Brazilian isolates of *Cylindrospermopsis raciborskii*

Caroline Hoff-Risetti<sup>1</sup>, Felipe Augusto Dörr<sup>2</sup>, Patrícia Dayane Carvalho Schaker<sup>1</sup>, Ernani Pinto<sup>2</sup>, Vera Regina Werner<sup>3</sup>, Marli Fátima Fiore<sup>1</sup>

<sup>1</sup>University of São Paulo, Center for Nuclear Energy in Agriculture, Piracicaba, Brazil; <sup>2</sup>University of São Paulo, Faculty of Pharmaceutical Sciences, São Paulo, Brazil; <sup>3</sup>Natural Sciences Museum, Zoobotanical Foundation of Rio Grande do Sul, Porto Alegre, Brazil

*Cylindrospermopsis raciborskii* has become one of the most studied species of cyanobacteria due to its frequent occurrence in freshwater blooms and its ability to produce cyanotoxins. In Brazil, *C. raciborskii* is the main species found in the freshwater bodies [1, 2]. The facts that the *C. raciborskii* population in Brazilian freshwater produces saxitoxin derivatives (STXs) [3] and that cylindrospermopsin (CYN) has not been detected so far in isolated strains, which is common in other countries [4] challenged us to investigate the occurrence of genes involved in CYN biosynthesis in the genomes of Brazilian *C. raciborskii* isolates. Five laboratory-cultured cyanobacterial strains were investigated for the presence of cylindrospermopsin synthetase (*cyr*) genes *cyrA*, *cyrB*, *cyrC* and *cyrJ*. A positive PCR amplification of *cyrA*, *cyrB* and *cyrC* genes was obtained for three strains (CENA302, CENA303 and CENA306). The sequences of the *cyrA*, *cyrB* and *cyrC* gene fragments obtained for CENA302 and CENA303 strains were translated to amino acids and the predicted protein functions and domains confirmed their identity as CYN synthetase genes. Also, phylogenetic analysis grouped the *CyrA*, *CyrB* and *CyrC* sequences together with their homologues found in two known CYN synthetase clusters (*C. raciborskii* strain AWT205 and CS-505) with high bootstrap support. Besides *cyr* genes, PCR products of the *sxtA*, *sxtB* and *sxtI* genes involved in STX production were also obtained. The translated amino acids and phylogenetic analysis identified the sequences as STX synthetase genes. Extensive LC-MS analyses were unable to detect CYN in isolated strains whereas the production of STX, GTX2 and GTX3 was confirmed in CENA302. The taxonomical positions of the five Brazilian *C. raciborskii* strains were confirmed by a detailed morphological description and a phylogenetic analysis of 16S rRNA gene sequences. To our knowledge this is the first study reporting the presence of *cyr* genes in *C. raciborskii* Brazilian isolates and the simultaneous presence of the *sxt* and *cyr* genes in single *C. raciborskii* strains.

[1] Sant'Anna *et al.*, 2008. Review of toxic species of cyanobacteria in Brazil. *Algological Studies* 126: 251-265; [2] Figueredo and Giani, 2009. Phytoplankton community in the tropical lake of Lagoa Santa (Brazil): Conditions favoring a persistent bloom of *Cylindrospermopsis raciborskii*. *Limnologia* 39: 264-272; [3] Lagos *et al.*, 1999. The first evidence of paralytic shellfish toxins in the fresh water cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. *Toxicon* 37: 1359-1373; [4] Everson *et al.*, 2011. Extreme differences in akinete, heterocyte and cylindrospermopsin concentrations with depth in a successive bloom involving *Aphanizomenon ovalisporum* (Forti) and *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju. *Harmful Algae* 10: 265-276

## P187: Photosynthetic production of isoprenoids

Hiroshi Kiyota<sup>1,2</sup>, Michiho Ito<sup>4</sup>, Masami Hirai<sup>2,3</sup> and Masahiko Ikeuchi<sup>1,3</sup>

<sup>1</sup>Department of Biological Science, University of Tokyo, Tokyo, Japan; <sup>2</sup>Plant Science Center, RIKEN, Saitama, Japan; <sup>3</sup>CREST, JST, Saitama, Japan; <sup>4</sup>Division of Pharmaceutical Sciences, University of Kyoto, Kyoto, Japan

Isoprenoids are one of major groups of secondary metabolites and are constructed of C<sub>5</sub> isoprene units. It is used for multiple purposes, for example, perfume, medicines, materials, pigments and biofuels. These are manufactured by chemical synthesis in part, but most of them are extracted and purified from biomaterials such as barks, leaves, roots and essential oils of plants. Bioproduction of isoprenoids using *Escherichia coli* and *Saccharomyces cerevisiae* has been studied [1, 2]. Although metabolic engineering in these organisms achieved high productivity and yield, these microbes consume organic carbon source and emit carbon dioxide. These weak points may conflict with demand of food and promote global warming. So, it is urgent to develop methods to produce isoprenoids from only inorganic carbon source. It would be highly demanded to produce isoprenoids with phototrophic organisms from carbon dioxide directly. Some cyanobacteria highly feasible for genetic engineering and it is getting ascendant that metabolic engineering enables cyanobacteria to produce various biofuels such as alcohols, fatty acids and hydrogen photosynthetically [3]. Since cyanobacteria naturally produce carotenoids and phytol to support photosynthesis, they may be a potential platform for large scale production of various isoprenoids. But there is relatively few studies for production of isoprenoids [4]. It remains to be studied that photosynthetic production of isoprenoids achieve high efficiency.

To establish the methodology of photosynthetic production of isoprenoids, we constructed cyanobacteria that heterologously produce terpenes by introducing plant genes of monoterpene synthases into *Synechocystis* sp. PCC6803. We evaluated production and accumulation of volatile monoterpene in cells and bubbling gas phase with gas stripping method. The produced terpene stayed in cells at low level, but was mostly recovered in gas phase. The accumulation rate of terpene in the gas phase was nearly constant over the culture period, where the cell growth was linear to stationary phase. We will also report effects of culture conditions on its productivity.

[1] Martin, V.J.J., Pitera, D.J., Withers, S.T., Newman, J.D. & Keasling, J.D. Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. *Nature biotechnology* 21, 796-802 (2003); [2] Ro, D.-K. et al. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* 440, 940-3 (2006); [3] Quintana, N., Van der Kooy, F., Van de Rhee, M.D., Voshol, G.P. & Verpoorte, R. Renewable energy from Cyanobacteria: energy production optimization by metabolic pathway engineering. *Applied microbiology and biotechnology* (2011); [4] Bentley, F.K. & Melis, A. Diffusion-based process for carbon dioxide uptake and isoprene emission in gaseous/aqueous two-phase photobioreactors by photosynthetic microorganisms. *Biotechnology and bioengineering* 109, 100-9 (2012)



## P188: Extracellular polymeric substances (EPS) from the unicellular cyanobacterium *Cyanothece* sp. CCY 0110: conditions influencing their production and polymer characterization

Rita Mota<sup>1</sup>, Rodrigo Guimarães<sup>1</sup>, Zsófia Büttel<sup>1</sup>, Federico Rossi<sup>2</sup>, Giovanni Colica<sup>2</sup>, Carla J. Silva<sup>3</sup>, Carla Santos<sup>3</sup>, Luís Gales<sup>1,5</sup>, Andrea Zille<sup>1</sup>, Roberto De Philippis<sup>2</sup>, Sara B. Pereira<sup>1</sup>, and Paula Tamagnini<sup>1,4</sup>

<sup>1</sup>IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal; <sup>2</sup>Department of Agricultural Biotechnology, University of Florence, Florence, Italy; <sup>3</sup>CeNTI - Centro de Nanotecnologia e Materiais Técnicos, Funcionais e Inteligentes, Vila Nova de Famalicão, Portugal; <sup>4</sup>Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal; <sup>5</sup>ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

Many cyanobacterial strains can synthesize and secrete extracellular polymeric substances (EPS) that can remain associated to the cell or be released into the surrounding environment (RPS-released polysaccharides). The particular characteristics of the cyanobacterial EPS, namely the presence of 2 different uronic acids, sulphate groups and high number of different monosaccharides (usually 6 to 10), makes them promising for biotechnological applications such as the removal of heavy metals from polluted waters. For the successful implementation of heavy metal removal systems based on cyanobacterial EPS, it is necessary to unveil the pathways utilized by these organisms for EPS synthesis and export, and identify the physiological/environmental factors that influence the synthesis and/or characteristics of the polymers. Previously, an *in silico* analysis of cyanobacterial genome sequences was performed and a putative mechanism for last steps of EPS production and export was proposed<sup>1</sup>. The aims of this work are the identification of the conditions that promote EPS production and the characterization of the produced polymer. The marine N<sub>2</sub>-fixing unicellular cyanobacterium *Cyanothece* sp. CCY 0110 was chosen as model organism since several *Cyanothece* strains are reported as strong EPS producers and efficient in the removal of metal ions from aqueous solutions<sup>2,3</sup>. *Cyanothece*'s growth and EPS production were evaluated in different physiological/ environmental conditions. The results obtained showed that the amount of EPS is mainly related to the number of cells, rather than to the amount of EPS produced by each cell. Therefore, conditions that promote growth (e.g. aeration, presence of nitrate, light intensity: 50 µE m<sup>-2</sup> s<sup>-1</sup>) also increase the amount of EPS produced per litre of culture. Having in mind industrial applications, a 10-fold scale up was performed for the conditions that favor growth/EPS production. X-ray diffraction, thermogravimetric and differential scanning calorimetry analysis revealed that the polymer produced by *Cyanothece* is remarkably thermo stable and mainly of amorphous nature. Ion-exchange chromatography and FTIR spectroscopy revealed that RPS are composed by 10 different monosaccharides (including 2 uronic acids, 2 pentoses and 2 deoxyhexoses). Moreover, the presence of sulphate groups and peptide bonds were detected, highlighting the complexity of the polymer.

<sup>1</sup>Pereira et al (2009) FEMS Microbiol Rev 33:917-941; <sup>2</sup>De Philippis et al (1998) Appl Environ Microbiol 64:1130-1132; <sup>3</sup>Micheletti et al (2008) J Appl Microbiol 105:88-94

## P189: Nostoginosins, potential trypsin inhibitors discovered from *Nostoc* sp. strain FSN

Liwei Liu<sup>1</sup>, Jouni Jokela<sup>1</sup>, Bahareh Nowruzi<sup>2</sup>, David P. Fewer<sup>1</sup>, Matti Wahlsten<sup>1</sup>, Lyudmila Saari<sup>1</sup> and Kaarina Sivonen<sup>1</sup>

<sup>1</sup> University of Helsinki, Department of Food and Environmental Sciences, Division of Microbiology, P.O.Box 56, 00014 Helsinki, Finland, E-mail: liwei.liu@helsinki.fi; <sup>2</sup> Tarbiat Moallem University, Department of Biology, Faculty of Science, 49 Dr.Mofatteh Avenue, P.O.Box: 15815-3587. Postal Code: 15614, Tehran, IRAN. E-mail: bahareh.nowruzi@helsinki.fi

Cyanobacteria are a rich resource of secondary metabolites, which have a variety of bioactivities, including anti-fungal, anti-cancer and enzyme inhibiting activities. We discovered a new potential trypsin inhibitor with a novel chemical structure from *Nostoc* sp. strain FSN. LC-MS analysis indicated that this compound named nostoginosin was a peptide composed of three subunits, 2-hydroxy-4-(4-hydroxyphenyl) butanoic acid, L-iIe and argininal. Seven milligrams of the purified nostoginosin with the yield of 0.5 mg g<sup>-1</sup> freeze-dried cells were used in NMR analysis which confirmed the structure. Nostoginosin is similar to the trypsin inhibitors, spumigins and aeruginosins, which consist of four subunits, argininal, variable D-amino acid, 4-(4-hydroxyphenyl) butanoic acid and specific amino acid (methylproline in spumigins and Choi in aeruginosins)<sup>[1][2]</sup>. Six nostoginosin variants were identified which had variation in all three subunits. According to the trypsin binding assay, both the aldehyde versions and alcohol versions of nostoginosin could bind the trypsin.

[1] Fewer, D. P., Jokela J., Rouhiainen, L., Wahlsten, M., Koskenniemi, K., Stal, L.J. and Sivonen, K. (2009) The non-ribosomal assembly and frequent occurrence of the protease inhibitors spumigins in the bloom-forming cyanobacterium *Nodularia spumigena*. *Molecular Microbiology*, 73, 924-937; [2] Ishida, K., Okita, Y., Matsuda, H., Okino, T. and Murakami M. (1999) Aeruginosins, Protease Inhibitors from the Cyanobacterium *Microcystis aeruginosa*. *Tetrahedron*, 55, 10971-10988

## P190: The production of small tetrapeptide protease inhibitors in the bloom-forming cyanobacterium *Nodularia spumigena*

David P. Fewer, Jouni Jokela, Eeva Paukku, Matti Wahlsten, Leo Rouhiainen, Gonzalo V. Gomez-Saez, Julia Österholm, Kaarina Sivonen

Department of Food and Environmental Sciences, Division of Microbiology, University of Helsinki, P.O. Box 56, Viikki Biocenter (Viikinkaari 9), FIN-00014, Helsinki, Finland

*Nodularia spumigena* is a filamentous diazotrophic cyanobacterium that forms blooms in brackish water bodies around the world. This cyanobacterium produces a range of linear and cyclic protease inhibitors thought to be part of a chemical defense against grazers. Here we show that it also produces 13 new members of the aeruginosin family of protease inhibitors. The genome of *N. spumigena* CCY9414 contains a compact 18 kb aer gene cluster encoding a peptide synthetase with a reductive release mechanism which offloads the aeruginosins as highly reactive peptide aldehydes. In addition to aeruginosins we found a plethora of small cyclic and linear peptides which could be assigned to spumigin, nodulapeptin, and nodularin families. Binding assays show that the aeruginosins and spumigins both interact strongly with serine proteases suggesting that the production of variants may be adaptive. The aeruginosins and spumigins are structurally very similar but produced simultaneously using separate peptide synthetases. We uncovered evidence of crosstalk between the spumigin and aeruginosin biosynthetic pathways suggesting a mechanism by which new natural products may evolve in nature.



## P191: Common occurrence and non-ribosomal biosynthesis of the antifungal glycolipopeptides, hassallidins, in cyanobacteria

**Kaarina Sivonen, Johanna Vestola, Jouni Jokela, David P. Fewer, Tania Shishido, Matti Wahlsten, Hao Wang & Leo Rouhiainen**

Department of Food and Environmental Sciences, Division of Microbiology, P.O. Box 56, Viikki Biocenter, Viikinkaari 9, FIN-00014, University of Helsinki, Finland

Hassallidins are cyclic glycosylated lipopeptides which inhibit the growth of a series of important opportunistic human pathogenic fungi. Two hassallidin variants, isolated from an epilithic cyanobacterium *Hassalia* sp., were known before this study. Here we report the discovery of multiple new hassallidin variants from the bloom forming cyanobacterium *Anabaena* strain 90 through genome sequencing. Additional 17 *Anabaena* strains were also found to produce a series of hassallidin variants by LC-MS. The newly discovered hassallidins had variation in fatty acid length, had acetylated sugars and a variable amino acid. We proved that hassallidins are encoded by a non-ribosomal peptide synthetase gene cluster (57 kb) from *Anabaena* sp. 90. The present day culture no longer produces hassallidins due to a deletion in gene cluster but they could still be identified from cells prior to the deletion in 1998 and from an anabaenopeptilide synthetase gene mutant (*apdA*<sup>-</sup>) strain of *Anabaena* sp. 90 constructed in 1999. This work led also to the prediction and detection of hassallidins in many other cyanobacteria including two strains of the toxic bloom forming cyanobacterium *Cylindrospermopsis raciborskii* and a strain of *Nostoc* sp. 159. We were also able to show that all the newly discovered hassallidins exhibit antifungal activity against *Candida albicans* and *Aspergillus flavus*. Further studies are needed to reveal the ecological significance of the detected common occurrence of these antifungal compounds in cyanobacteria.

## P192: Detection of purple photosynthetic bacteria from leachates generated in Bordo Poniente Stage IV landfill from Mexico City

**<sup>1</sup>María Teresa Núñez-Cardona, <sup>1</sup>Magdalena Chávez Hernández, <sup>2</sup>Luis Raúl Tovar Gálvez, <sup>2</sup>María Eugenia Gutiérrez Castillo**

<sup>1</sup>UAM-X, Universidad Autónoma Metropolitana-Xochimilco; <sup>2</sup>CIEMAD-Centro Interdisciplinario de Investigaciones y Estudios sobre Medio Ambiente y Desarrollo

Photosynthetic bacteria are beneficial and efficient microorganisms because they are not pathogenic, and produce a great number of secondary metabolites that could be used both for human and environmental health. Herbicides, hormones, vitamins and growth factors that improve plant growth and increase plant yield are produced by phototrophic bacteria. Also, these bacteria could be used for wastewater treatment[1]. Leachates result of garbage decomposition in landfills and could be a source of beneficial microorganisms but actually this material is not used for this purpose. We collected four samples from macrocells where leachates are retained at the landfill Bordo Poniente, Stage IV (BPS-IV), and they were used for enriching a liquid medium containing: 1000 mL distilled water, 5.0g KH<sub>2</sub>PO<sub>4</sub>; 3.0g MgSO<sub>4</sub>•7H<sub>2</sub>O, 0.4 g NH<sub>4</sub>Cl, 0.05 g CaCl<sub>2</sub>•2H<sub>2</sub>O; 0.4 g de NaCl, 1.0 succinate; B<sub>12</sub> vitamin (2.0 mg/100 mL distilled water). 1.0 FeCl<sub>3</sub> (1.6 g/1000 ml distilled water), and 1.0 ml van Niel's solution[3]. Culture incubation where done by incandescent light (1800 lux), at ambient temperature and anaerobic conditions. After 15 days in these conditions, cultures were red and brown in colors; this is the first evidence of the photosynthetic bacteria growth in leachates from the landfill BPS-IV. Using glycerol as supporting agent, pigment *in vivo* analyses showed the presence of bacteriochlorophyll *a* in 375-379, 588-589, 802-804, 856-858 nm and carotenes in: 456, 494-496 and 594 nm. Methanol pigment extracts had maximal absorption at 364 and 771 nm for bacteriochlorophyll *a* and 428, 452, 467, 473, 461-468 nm for carotenes. Leachates from the BPS-IV could be a source of beneficial microorganisms like anoxygenic photosynthetic sulfur and nonsulfur bacteria.

[1]Getha K, Vikineswary S, Chong VC 1998, Isolation and growth of the phototrophic bacterium *Rhodospseudomonas palustris* strain B1 in sago-starch-processing wastewater, World Journal of Microbiology and Biotechnology 14 (4):505-511; [2]van Niel CB, 1971. Techniques for the enrichment, isolation and maintenance of the photosynthetic bacteria. A. San Pietro (Ed.), Methods in Enzymology, Academic Press, London, pp. 3-28

## P193: High Cell Density Cultivation of *Cyanothece* sp. ATCC 51142

**Swathi Alagesan<sup>1</sup>, Sandeep B. Gaudana<sup>1</sup>, Subbiah Krishnakumar<sup>1</sup>, Pramod P. Wangikar\*<sup>1</sup>**

<sup>1</sup>Department of Chemical Engineering, Indian Institute of Technology Bombay, India; \*wangikar@iitb.ac.in

In our present work, we report a model based optimization for high cell density cultivation of *Cyanothece* sp. ATCC 51142. It has been reported in literature that *Cyanothece* display mixotrophic growth [3]. Through preliminary flask experiments, the optimum Cyanobacteria find widespread application in food and beverage industry, as biofertilizers and, in lipid and biofuel production. They are unicellular photosynthetic bacteria which can sequester atmospheric carbon dioxide and convert them into various commercially useful products [2]. *Cyanothece*, a species of cyanobacteria, is also capable of fixing atmospheric nitrogen thereby producing hydrogen as a byproduct [1]. Hence, these bacteria fix atmospheric carbon dioxide and store them as energy reserves, which can be utilized for energy intensive processes like nitrogen fixation and hydrogen production. Thus, they are capable of not only producing industrially important products, but also tackling environmental issues like carbon dioxide mitigation and energy production, emphasizing their importance as an excellent "single cell factory". However for *Cyanothece* species to become commercially viable, high cell density cultivation of the organism is required. The success of high cell density cultivation relies upon the ability to control and maintain the different growth parameters including various media components such that they neither inhibit nor become limiting. In our present work, we report a model based optimization for high cell density cultivation of *Cyanothece* sp. ATCC 51142. It has been reported in literature that *Cyanothece* display mixotrophic growth [3]. Through preliminary flask experiments, the optimum concentration of various media components was obtained. It was also observed that in a batch culture the growth saturates after the media components get exhausted. Hence a fedbatch mode of cultivation was adopted wherein the feeding strategy of various nutrients was optimized. The final biomass obtained under such conditions was nine folds higher than that reported in literature. A mathematical representation of the process was formulated in order to optimize the growth parameters. Such a model based optimization for high cell density cultivation of *Cyanothece* species is crucial for its commercial application and can also be adopted for other strains.

[1] Bandyopadhyay A. et al., Nature Communications, 2011; [2] Reddy K. J et al., Journal of Bacteriology, 1993; [3] Schneegurt M.A. et al., Journal of Phycology, 1997

**P194: Exploitation of stable labelling for evaluation of *de novo* microcystin production****Christine Edwards (c.edwards@rgu.ac.uk), Carlos J. Pestana (c.j.pestana@rgu.ac.uk) and Linda A. Lawton (l.lawton@rgu.ac.uk)**

IDEAS Research Institute, The Robert Gordon University, St. Andrew Street, Aberdeen, AB25 1HG, UK

Microcystins are a large group of hepatotoxic peptides produced by multiple genera of cyanobacteria, which often form prolific blooms posing a threat to human and animal health.

The role of microcystins is still unclear and although well studied, most reports present data where there is no clear differentiation between existing and newly synthesized peptide. In order to gain greater understanding on interactions of microcystins and other bioactive peptides we used the incorporation of  $^{15}\text{N}$  using media supplemented with  $^{15}\text{N}$   $\text{NaNO}_3$  for growth of *Microcystis aeruginosa* where growth was based on cell numbers using flow cytometry and *de novo* microcystins were quantified by LC-MS.

Completely labeled microcystin-LR was detected within 48 hours and provided an opportunity to study the effects of different parameters in much shorter experiments than the typical 4-6 weeks. This methodology was used to study the effects of temperature, extracellular microcystin-LR, cell density and spent media from *Planktothrix agardhii* CYA 29 on true microcystin production in *M. aeruginosa*. Data from all experiments confirmed that this approach was rapid and reliable with many potential applications. In addition, the evidence of rapid synthesis of labeled microcystin supports the need to redefine the definition of secondary metabolite.

Engelke, C.J., Lawton, L.A. and Jaspars, M. (2003) Elevated microcystin and nodularin levels in cyanobacteria growing in spent medium of *Oscillatoria agardhii*. *Archiv für Hydrobiologia*, 158, 541-550

**P196: Electron transfer in the reaction center of *Heliobacterium modesticaldum* in vivo and in vitro****Kevin E. Redding<sup>1,3</sup>, Iosifina Sarrou<sup>1</sup>, John Cowgill<sup>1</sup>, Su Lin<sup>1,2</sup>, Fabrice Rappaport<sup>3</sup>, Trevor Kashey<sup>1</sup>, Steven Romberger<sup>4</sup>, and John H. Golbeck<sup>4</sup>**

<sup>1</sup>The Department of Chemistry & Biochemistry and <sup>2</sup>The Biodesign Institute, Arizona State University, Tempe AZ 85287-1604; <sup>3</sup>Institut de Biologie Physico-Chimique, UMR 7141 CNRS-Univ. P. et M. Curie, 75005 Paris, France; <sup>4</sup>Department of Biochemistry and Molecular Biology, and Department of Chemistry, The Pennsylvania State University, University Park, PA 16801

Heliobacteria contain a very simple photosynthetic apparatus, consisting of a homodimeric type I reaction center (RC) without a peripheral antenna system and using the unique pigment bacteriochlorophyll (BChl) *g*. They are thought to use a light-driven cyclic electron transport pathway to pump protons, and thereby phosphorylate ADP. We previously reported that the fluorescence emission from the heliobacterial RC *in vivo* was increased by exposure to actinic light, although this variable fluorescence phenomenon exhibited very different characteristics to that in oxygenic phototrophs. Here we describe the underlying mechanism behind the variable fluorescence in heliobacterial cells. We find that the ability to stably photobleach  $\text{P}_{800}\text{O}$ , the primary donor of the RC, using brief flashes is inversely correlated to the variable fluorescence. Using pump-probe spectroscopy in the nanosecond timescale, we found that illumination of cells with bright light for a few seconds put them into a state in which a significant fraction of the RCs would back-react from the  $\text{P}_{800}^+\text{A}_0^-$  state within 10-20 ns. The fraction of RCs in the back-reacting state correlated with the variable fluorescence, indicating that nearly all of the increase in fluorescence could be explained by charge recombination of  $\text{P}_{800}^+\text{A}_0^-$ , some of which regenerated the excited state. This hypothesis was tested directly by time-resolved fluorescence studies in the ps and ns timescales. The major decay component in cells had a 20-ps decay time, representing trapping by the RC. Treatment with dithionite resulted in the appearance of a ~18-ns decay component, which accounted for ~0.5% of the decay, but was undetectable in the untreated cells.

We have also purified the RC from this species to homogeneity. It is composed of a PshA homodimer and lacks the PshB polypeptide binding the  $\text{F}_\text{A}/\text{F}_\text{B}$  [4Fe-4S] clusters. The stoichiometry of cofactors within the HbRC was determined to be  $19 \pm 3$  BChl *g* : 2 BChl *g'* : 2  $8^1\text{-OH-Chl } a_f$  : 1 4,4'-diaponeurosporene : 1.6 menaquinone (7:1 ratio of MQ-9:MQ-8). We suggest that the  $\text{F}_\text{x}$  [4Fe-4S] interpolypeptide cluster is actually the terminal acceptor, as it can reduce exogenous electron carriers in the absence of PshB. The  $\text{F}_\text{x}$  cluster can be reduced with dithionite at high pH, as evidenced by the lack of stable  $\text{P}_{800}$  photobleaching and the appearance of the  $\text{F}_\text{x}^-$ -EPR signal. This indicates that the  $\text{F}_\text{x}$  cluster of the heliobacterial RC has a much higher reduction potential than FX in Photosystem I. Pump-probe analysis in the ps timescale reveals a trapping time of ~20 ps to generate  $\text{P}_{800}^+\text{A}_0^-$  and a ~700-ps reoxidation of  $\text{A}_0^-$ ; the latter can be blocked by treatment with dithionite at high pH. As seen previously in other species, we find no evidence for a role of the MQ in electron transfer within the RC. In conclusion, the heliobacterial RC is very different from Photosystem I in oxygenic phototrophs.

Supported by DOE grant DE-FG02-98ER20314 MOD 012

# PARTICIPANTS LIST



# LIST OF PARTICIPANTS

<i>Aaron Kaplan</i> .....	OC13; OC15; P26
<i>Aaron Setterdahl</i> .....	OC7
<i>Adrian Kölsch</i> .....	P118
<i>Afef Fathalli</i> .....	P16; P28
<i>Aleksandra Kapuscik</i> .....	P164; P178
<i>Alicia M. Muro-Pastor</i> .....	P31; P154
<i>Amanda Cockshutt</i> .....	P56
<i>Amin Nasser</i> .....	P154
<i>Ana Luisa Pereira</i> .....	P2
<i>Ana Maria Sánchez Riego</i> .....	P126
<i>Ana Paula Dini Andreote</i> .....	P3
<i>Ana Valladares Ruiz</i> .....	OC24; OC44; P138
<i>Andreia da Anunciação Gomes</i> .....	P17
<i>Andreia Verissimo</i> .....	OC47
<i>Ângela Brito</i> .....	P185
<i>Anika Wiegard</i> .....	P105
<i>Ankita Kothari</i> .....	P127
<i>Ann Magnuson</i> .....	P80
<i>Anne Ylinen</i> .....	P18; P165; P182
<i>Annegret Wilde</i> .....	P32; P77; P79; P101; P105; P148
<i>Anthony Dron</i> .....	P106; OC17
<i>Anton Liaimer</i> .....	P166
<i>Antonia Herrero</i> .....	OC24; OC44; OC52; P33; P64; P65; P75; P147
<i>Antonio López-Lozano</i> .....	P33
<i>Arjan Vermeulen</i> .....	P107
<i>Arnaud Taton</i> .....	OC10
<i>Ayumi Kizawa</i> .....	P34; P93
<i>Beate Heilmann</i> .....	P128
<i>Birgitta Norling</i> .....	P121; P129; P151

<i>Bogdan Druga</i> .....	P35
<i>Brandon S. Guida</i> .....	P4
<i>Brett Neilan</i> .....	PL12
<i>C Peter Wolk</i> .....	
<i>Camila Francieli da Silva Malone</i> .....	P5
<i>Carl Bauer</i> .....	P104; P107; P116; P162
<i>Carla Trigo</i> .....	P36; P98
<i>Carolina Nishi</i> .....	P108
<i>Caroline Autenrieth</i> .....	P119
<i>Caroline Hoff Riseti</i> .....	P186
<i>Catarina Churro</i> .....	P6
<i>Catarina Pacheco</i> .....	OC30; OC41; P103; P125
<i>Charlotte de Araujo</i> .....	P37; P72
<i>Cheng-Cai Zhang</i> .....	P38
<i>Cheryl Kerfeld</i> .....	PL14; P109
<i>Chihiro Azai</i> .....	P130; P133
<i>Christine Edwards</i> .....	P22; P194
<i>Ciprian Chis</i> .....	P35
<i>Conrad Mullineaux</i> .....	OC9; OC37; P40; P111
<i>Cosmin ionel Sicora</i> .....	P35
<i>Cristiana Moreira</i> .....	OC4; P16; P28
<i>Csaba István Nagy</i> .....	OC16
<i>Daniela Ferreira</i> .....	PL18; OC27; P125
<i>David B. Knaff</i> .....	OC8
<i>David Encinas Pisa</i> .....	OC44; P155
<i>David Fewer</i> .....	OC31; P172; P182; P183; P189; P190; P191
<i>David Scanlan</i> .....	PL5
<i>Davide Zannoni</i> .....	P167
<i>Debashish Bhattacharya</i> .....	PL2
<i>Denis Jallet</i> .....	P39
<i>Dennis Nürnberg</i> .....	P40



<i>Diana Kirilovsky</i> .....	P39; P74
<i>Dinka Mandakovic</i> .....	P36; P98
<i>Donald Bryant</i> .....	PL4; OC14; OC28; OC29; OC53; P20
<i>Douglas Risser</i> .....	PL18; P42
<i>Dzmitry Haurusev</i> .....	P43
<i>Edward Spence</i> .....	P111; P124
<i>Eiji Suzuki</i> .....	P44
<i>Eitan Salomon</i> .....	OC51; P29
<i>Elisabete Valério</i> .....	P6
<i>Eliška Zapomělová</i> .....	OC2; P1; P171
<i>Elke Dittmann</i> .....	OC13
<i>Enrique Flores</i> .....	OC24; OC44; OC52; P33; P40; P64; P65; P75; P98; P138; P147
<i>Erina Kobe</i> .....	P131
<i>Estelle Couradeau</i> .....	P7
<i>Éva Kiss</i> .....	P160
<i>Eva-Mari Aro</i> .....	PL19; OC46; P53; P69; P71; P136; P170
<i>Evan W. Roberts</i> .....	P109
<i>Fabian Billenkamp</i> .....	P45
<i>Fernanda Rios Jacinavicius</i> .....	P46
<i>Fernando de la Cruz</i> .....	OC44; P155
<i>Ferran Garcia-Pichel</i> .....	PL17; P4; P127
<i>Fevzi Daldal</i> .....	OC26; OC47
<i>Filipe Pinto</i> .....	OC30; OC41; P103; P125;
<i>Florian Humily</i> .....	P47
<i>Francy El Souki</i> .....	P156
<i>Friederike Klemke</i> .....	P110
<i>Fumihito Itoh</i> .....	P48
<i>Gabriele Klug</i> .....	PL7; P45
<i>Gaozhong Shen</i> .....	
<i>Gen Enomoto</i> .....	P49; P73
<i>Georg Schmetterer</i> .....	OC48; P138

George Owtrim.....	P99
George S Espie.....	P37; P72
Gergely Lakatos.....	P132
Ghada Ajlani.....	P154
Giovanni Sandrini.....	P50
Guilherme Scotta Hentschke.....	P8
Guntram Christiansen.....	P168; P180
Guogang Dong.....	OC10
Guo-Shu Wang.....	P51
Hagai Raanan.....	P26
Hagit Zer.....	OC51
Hajime Masukawa.....	OC20; P169
Hanna Sipari.....	P18; P165
Hannu Leino.....	P71; P170
Hans C.P. Matthijs.....	OC19; P50
Haruki Yamamoto.....	P52
Helena M. Galvão.....	P27
Henna Silén.....	OC46; P53; P69
Hidehiro Sakurai.....	OC20; P169
Himadri Pakrasi.....	PL20
Hirofumi Yoshikawa.....	P145; P150; P159
Hiroshi Kiyota.....	P187
Hirozo Oh-oka.....	P130; P133
Hugo Scheer.....	PL1
Igor Stadnichuk.....	OC43
Imre Vass.....	OC16; OC23; P134; P152; P160
Iris Maldener.....	P117
Isabel Esteve.....	P184
Iwane Suzuki.....	OC11; P48; P54
J Thomas Beatty.....	P100
Jack Meeks.....	OC27; P42; PL18

Jackie Zorz.....	P56
James W Golden.....	OC10; P57
Jan Mares.....	P9; P171
Jana Knoppová.....	P135
Jari Kämäräinen.....	P136
Javier Espinosa.....	P122
Jean Huang.....	OC5
Jens Appel.....	OC25; P115
Joana Gaifem.....	P185
Joanna Sacharz.....	P111
Joaquin Giner Lamia.....	P58
Jogadhenu Prakash.....	OC11
Johannes F. Imhoff.....	P13
Jonathan Zehr.....	PL16
Jörg Overmann.....	PL15
Josef Komenda.....	P59; P135; P137; P140; 141
Juan Maldonado.....	P184
Julia Holtzendorff.....	P101
Julia Österholm.....	P165; P172; P190
Julian Eaton-Rye.....	OC45
Kaarina Sivonen.....	OC31; P18; P19; P165; P170; P172; P182; P183; P189; P190; P191
Kaisa Hakkila.....	P60; P66; P89
Kaisa Haukka.....	P19
Kaori Inoue-Sakamoto.....	P123
Kaori Ohki.....	P24; P52; P173
Karin Stensjö.....	OC54
Karoline Schreiber.....	
Kathryn Fixen.....	
Kazuki Terauchi.....	P61
Kevin Redding.....	P196
Kinga Klodawska.....	P55

<i>Kirstin Gutekunst</i> .....	P115
<i>Klaus Benedikt Möllers</i> .....	OC34
<i>Kleber Renan de Souza Santos</i> .....	P62
<i>Kristina Felcmanova</i> .....	P63
<i>Laura Corrales Guerrero</i> .....	P64
<i>Lena Gerwick</i> .....	PL10; P174
<i>Leo Rouhiainen</i> .....	P172; P190; P191
<i>Leticia Escudero Crujera</i> .....	P65
<i>Lifang Zhang</i> .....	P121; P129; P151
<i>Liisa Gunnelius</i> .....	P66; P89
<i>Linda Vuorijoki</i> .....	P67
<i>Liwei Liu</i> .....	P189
<i>Loralyn Cozy</i> .....	P68
<i>Louis Sherman</i> .....	OC40
<i>Luca Bersanini</i> .....	P69; P53
<i>Lucas J. Stal</i> .....	OC12
<i>Luciana Retz de Carvalho</i> .....	P46; P175
<i>Mai Watanabe</i> .....	P70
<i>Marcus Ludwig</i> .....	PL4; OC28; OC29
<i>Marcus Tank</i> .....	P20
<i>Margarida Costa</i> .....	P176; P177; P179
<i>Maria Ermakova</i> .....	P71
<i>Maria Estela Stenico</i> .....	
<i>Maria João Ribeiro</i> .....	P176; P177
<i>María Teresa Núñez Cardona</i> .....	P15; P192
<i>Marion Eisenhut</i> .....	OC46; P53
<i>Mark Gomelsky</i> .....	PL6
<i>Markéta Bohunická</i> .....	P9
<i>Marketa Foldynova</i> .....	P137
<i>Markus Mikulic</i> .....	OC48; P138
<i>Marta Vaz Mendes</i> .....	P185

<i>Martin Hagemann</i> .....	OC13; OC38
<i>Martin Mulligan</i> .....	
<i>Martin Tichy</i> .....	P59; P137; P139; P140; P141
<i>Martina Beckova</i> .....	P140
<i>Maryam Moazami-Goudarzi</i> .....	P72
<i>Masahiko Ikeuchi</i> .....	P49; P70; P73; P82; P95; P150; P159; P187
<i>Matthias Futschik</i> .....	P157; P158
<i>Michael Madigan</i> .....	PL3
<i>Michael Summers</i> .....	OC36
<i>Michal Gwizdala</i> .....	P39; P74
<i>Michal Koblížek</i> .....	OC1; P12; P43; P97
<i>Miguel A. Hernández-Prieto</i> .....	P157; P158
<i>Miguel Lopo</i> .....	P102; P103
<i>Min Chen</i> .....	OC42; P160
<i>Mireia Burnat</i> .....	P65; P75; P184
<i>Mitsumasa Hanaoka</i> .....	P76
<i>Mónica Vásquez</i> .....	P36; P98
<i>Muriel Gugger</i> .....	PL14; P44
<i>Nataliya Velichko</i> .....	P14
<i>Neil Hunter</i> .....	PL9
<i>Néstor Cortez</i> .....	
<i>Niels-Ulrik Frigaard</i> .....	PL4; OC34; OC53
<i>Nils Schürgers</i> .....	P32; P77; P79
<i>Nir Keren</i> .....	OC51; P26; P29; P118
<i>Oana Sicora</i> .....	P35
<i>Oleksandra Fokina</i> .....	P78
<i>Olga Siadat</i> .....	P79
<i>Olivier Ploux</i> .....	OC22
<i>Otakar Strunecky</i> .....	P41
<i>Patrícia Raleiras</i> .....	P80
<i>Patrick Videau</i> .....	P112

<i>Paul Janssen</i> .....	P10
<i>Paula Tamagnini</i> .....	OC21; OC30; OC41; P102; P103; P125; P185; P188
<i>Paulo Oliveira</i> .....	OC54; P103
<i>Pavel Hrouzek</i> .....	P164; P171; P178
<i>Pedro Leão</i> .....	P176; P177; P179
<i>Peter Kós</i> .....	OC16; OC23; P160
<i>Peter Lindblad</i> .....	PL11; P80
<i>Peter Nixon</i> .....	PL21; OC37; P135; P140
<i>Petra Skotnicova</i> .....	P141
<i>Philipp Savakis</i> .....	P32; P113
<i>Pia Lindberg</i> .....	OC18
<i>Pin-Ching Maness</i> .....	P143
<i>Qian Dong</i> .....	P104
<i>Rainer Kurmayer</i> .....	P168; P180
<i>Ralf Steuer</i> .....	P118; P144
<i>Raymond Cox</i> .....	OC53
<i>Refat Abdel-Basset</i> .....	P49; P70; P73; P81; P95; P159
<i>Rei Narikawa</i> .....	P49; P70; P73; P82; P95; P179
<i>Rina Aoki</i> .....	P83
<i>Rita Mota</i> .....	OC21; P188
<i>Robert A. Niederman</i> .....	OC49
<i>Roberto De Philippis</i> .....	OC21; PL13; P188
<i>Robin Ghosh</i> .....	P51; P119
<i>Rosário Martins</i> .....	P25; P176; P177; P179
<i>Rosmarie Rippka</i> .....	
<i>Rüdiger Schulz</i> .....	OC25; P115
<i>Ryoma Tsujimoto</i> .....	P84
<i>Ryudo Ohbayashi</i> .....	P145; P159
<i>Samantha Bryan</i> .....	OC9; OC37; P111
<i>Sandra Díaz Troya</i> .....	P85
<i>Sara Bernardes Pereira</i> .....	OC21; P188



<i>Sara Iverson</i> .....	
<i>Satomi Iwata</i> .....	P21
<i>Satoru Watanabe</i> .....	P145; P150; P159
<i>Sébastien Zappa</i> .....	P162
<i>Seiji Akimoto</i> .....	P131; P146
<i>Sergio Camargo</i> .....	OC24
<i>Sérgio Ferreira</i> .....	P103
<i>Shaista Hameed</i> .....	P22
<i>Shigeki Ehira</i> .....	P86
<i>Shigeru Itoh</i> .....	P87
<i>Shin-ichi Maeda</i> .....	OC39
<i>Shinichi Takaichi</i> .....	P181
<i>Shinji Masuda</i> .....	P153 /P120
<i>Shuyi Zhang</i> .....	OC14
<i>Silvia Picossi Goñi</i> .....	P147
<i>Simeoni Iuliana</i> .....	
<i>Sofia Costa</i> .....	P11
<i>Sophie Rabouille</i> .....	OC17; P106
<i>Steeve Lima</i> .....	
<i>Susan Cohen</i> .....	P88
<i>Susan Golden</i> .....	OC10; OC55; P88
<i>Suvi Känä</i> .....	P182
<i>Swathi Alagesan</i> .....	P193
<i>Taina Tyystjärvi</i> .....	P60; P66; P89
<i>Takashi Osanai</i> .....	P114
<i>Tânia Keiko Shishido</i> .....	P183; P191
<i>Tanya Soule</i> .....	OC35
<i>Taro Kadowaki</i> .....	P90
<i>Teresa Thiel</i> .....	OC32
<i>Thomas Wallner</i> .....	P79; P148
<i>Thorsten Heidorn</i> .....	

<i>Tiago Guerra</i> .....	OC28
<i>Tiago Toscano Selão</i> .....	P121; P129; P151
<i>Tina Summerfield</i> .....	P91
<i>Toivo Kallas</i> .....	OC6
<i>Tomáš Hauer</i> .....	P23
<i>Toshio Sakamoto</i> .....	OC33; P123
<i>Verena Schön</i> .....	P161; P158
<i>Veronica Carbonell</i> .....	P149
<i>Vicente Mariscal Romero</i> .....	OC52; P64; P65
<i>Vitor Vasconcelos</i> .....	PL22; OC4; P2; P6; P11; P16; P28; P176; P177; P179; P185
<i>Vladimir Gorlenko</i> .....	OC3
<i>Wei Huang</i> .....	
<i>William Gerwick</i> .....	PL10; P174; P179
<i>Wojciech Bialek</i> .....	
<i>Wolfgang Hess</i> .....	PL8; OC46; P31; P77; P99; P158; P161
<i>Yonghui Zeng</i> .....	P12
<i>Yoshitaka Saga</i> .....	P163
<i>Yu Kanesaki</i> .....	P145; P150; P159
<i>Yuichi Fujita</i> .....	P52; P92; P83; P84; P94
<i>Yuka Watanabe</i> .....	
<i>Yukako Hihara</i> .....	P34; P90; P93
<i>Yukiko Taniuchi</i> .....	P24
<i>Yuto Hiraide</i> .....	P94
<i>Yuu Hirose</i> .....	P95
<i>Yvonne Zilliges</i> .....	OC13; P96
<i>Zahra Choolaei</i> .....	OC50 / P30
<i>Zsofia Buttel</i> .....	OC30; P188
<i>Zsuzsanna Deák</i> .....	P152; P134
<i>Zuzana Vávrová</i> .....	P97

# PROGRAM

	SUNDAY, 5	MONDAY, 6	TUESDAY, 7	WEDNESDAY, 8	THURSDAY, 9	FRIDAY, 10
8:30						
9:00		SESSION I: PLENARY PHYLOGENY, TAXONOMY, AND DIVERSITY   ROOM: PORTO	SESSION III PLENARY: PHYSIOLOGY, METABOLISM, SENSING AND SIGNAL TRANSFER   ROOM: PORTO	SESSION V PLENARY: BIOREMEDIATION, SECONDARY METABOLITES AND OTHER APPLIED ASPECTS   ROOM: PORTO	SESSION I PLENARY: PHYLOGENY, TAXONOMY AND DIVERSITY   ROOM: PORTO	SESSION III PLENARY: PHYSIOLOGY, METABOLISM, SENSING AND SIGNAL TRANSFER   ROOM: PORTO
9:30		Coffee break	Coffee break	Coffee break	Coffee break	Coffee break
10:00		SESSION IV: ORAL COMMUNICATIONS   ROOM: PORTO	SESSION IV: ORAL COMMUNICATIONS   ROOM: PORTO	SESSION V PLENARY: BIOREMEDIATION, SECONDARY METABO- LITES AND OTHER APPLIED ASPECTS   ROOM: PORTO	SESSION IV: ORAL COMMUNICATIONS   ROOM: PORTO	SESSION III: ORAL COMMUNICATIONS   ROOM: PORTO
10:30						
11:00		SESSION I: ORAL COMMUNICATIONS   ROOM: DOURO				
11:30			Lunch	Lunch	Lunch	Lunch
12:00						
12:30			Lunch	Lunch	Lunch	Lunch
13:00			Scientific International Committee meeting   ROOM: CORGO - FLOOR 2			
13:30				SESSION III: ORAL COMMUNICATIONS   ROOM: PORTO		
14:00		SESSION V: ORAL COMMUNICATIONS   ROOM: PORTO	POSTER SESSION   FOYER AND ROOM 3 RIOS	SESSION V: ORAL COMMUNICATIONS   ROOM: DOURO	POSTER SESSION II   FOYER AND ROOM 3 RIOS	SESSION IV PLENARY: BIOENERGETICS, PROTEOMICS AND GENOMICS   ROOM: PORTO
14:30						
15:00				Coffee break		
15:30				Coffee break	Coffee break	Coffee break
16:00	Registration	Coffee break	Coffee break	SESSION IV: ORAL COMMUNICATIONS   ROOM: PORTO	Coffee break	Coffee break
16:30		SESSION II PLENARY: ENVIRONMENT AND ECOLOGY   ROOM: PORTO	SESSION IV PLENARY: BIOENERGETICS, PROTEOMICS AND GENOMICS   ROOM: PORTO		SESSION II PLENARY: ENVIRON- MENT AND ECOLOGY   ROOM: PORTO	CLOSING LECTURE   ROOM: PORTO
17:00				Sightseeing tour		Closing session
17:30	Welcome					
18:00	OPENING LECTURE   ROOM: PORTO					
18:30						
19:00	Reception					
19:30						
20:00				Dinner - Círculo Universitário		
20:30						