Book of Abstracts
8th Congress of the International Symbiosis Society

{Symbiotic Lifestyle}

Venue & Date: Faculdade de Ciências da Universidade de Lisboa; Lisbon, Portugal; 12-18 July, 2015
Editors: Silvana Munzi, Florian Ulm
Organizing Committee: Silvana Munzi, Cristina Cruz, Rusty Rodriguez, Irene Newton

Welcome!

Held every three years and organized by the International Symbiosis Society, the Congress is focused on a concept - symbiosis. Long viewed as an exception, a curiosity on the margins of biology, symbiosis is today considered ubiquitous and one of the main characteristics of the biological systems. The University of Lisbon (ULisboa; http://www.ulisboa.pt/) was created in 2013 based on the union of university institutions, which date back to the 13th century. We hope that this symbiosis between new and old will create the perfect environment to host the congress “Symbiosis 2015”. We welcome all researchers, educators, and students who work in the many diverse fields which involve symbioses. The theme of the congress is Symbiotic Lifestyle with presentations spanning a continuum from molecules to ecosystems, encompassing plants, animals and microbes of all genre. The breadth of abstracts is a testimonial to the ubiquity and significance of symbiosis. An electronic version of abstracts is provided for the 8th International Symbiosis Society Congress. Please enjoy the abstracts, which can be searched within the document and are linked with the index, as you participate in a congress addressing one of the most fundamental aspects of plant and animal life on this precious planet. We hope that this meeting will be an ideal venue for discussion, exchange and transfer of knowledge, helping to create new and foster existing collaborations in “Symbiotic lifestyle” between researchers.

We hope you enjoy.

The organizing committee

Cristina Cruz

Rusty Rodriguez	Cristina Cruz	Silvana Munzi

Scientific & Organizing Committee, Chairs

Scientific committee:

Silvana Munzi (Faculdade de Ciências da Universidade de Lisboa)
Cristina Cruz (Faculdade de Ciências da Universidade de Lisboa)
Rusty Rodriguez (Adaptive Symbiotic Technologies)

Organizing committee:

Silvana Munzi (Faculdade de Ciências da Universidade de Lisboa)
Cristina Cruz (Faculdade de Ciências da Universidade de Lisboa)
Rusty Rodriguez (Adaptive Symbiotic Technologies)
Irene Newton (Indiana University)

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Flore Zélé (Universidade de Lisboa, Portugal)
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Irene L.G. Newton (Indiana University, USA)
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Jan Sapp (York University, Canada)
Jillian M. Petersen (Max Planck Institute, Germany)
Karin Pritsch (Helmholtz Zentrum Muenchen, Germany)
Katarzyna Turnau (Jagiellonian University, Poland)
Luis Carvalho (Universidade de Lisboa, Portugal)
Luis Lopez Llorca (Universidad de Alicante, Spain)
Luis Teixeira (Instituto Gulbenkian de Ciência, Portugal)
Manju M. Gupta (University of Delhi, India)
Manuela Giovannetti (Università di Pisa, Italy)
Marc-André Selosse (Muséum National d'Histoire Naturelle, France)
Maria J.Pozo (Consejo Superior de Investigaciones Científicas, Spain)
Martin Kaltenpoth (Max Planck Institute for Chemical Ecology, Germany)
Nathalie Gontier (Universidade de Lisboa, Portugal)
Patricia Stock (University of Arizona, USA)
Rusty J. Rodriguez (Adaptive Symbiotic Technologies, USA)
Sara Magalhães (Universidade de Lisboa, Portugal)
Silvana Munzi (Universidade de Lisboa, Portugal)
Stanley Freeman (The Volcani Center, Israel)
Thorsten Grams (Technische Universitaet Muenchen, Germany)
William B. Sanders (Florida Gulf Coast University, USA)

Practical information

Conference Venue:

Faculdade de Ciências da Universidade de Lisboa

Campo Grande 1749-016 Lisboa

Wireless (at the conference venue):

Username: symbios@alunos.fc.ul.pt

Password: Conf#2015

Contacts:

Madalena Almeida: - Viagens Abreu, S.A. (RNAVT 1702)
Av. 25 de Abril, 2 | 2799-556 Linda-a-Velha | Portugal
Tel: +351 21 415 6121 | Fax: +351 21 415 6383
Email: madalena.almeida@abreu.pt

Silvana Munzi: +351 910759309

Cristina Cruz: +351 964880356

Radio taxi Lisboa: +351 218119000

Taxis de Lisboa: +351 21 936 21 13

Emergency number: 112

Symbiosis 2015 at a glance

Sun 12	**Mon** 13	**Tue** 14	**Wed** 15	**Thur** 16	**Fri** 17
	8:30- 9:00 **Opening**		**Cultural day**		
	9:00 - 11:00 **SLS** 9:00 - 11:00 **EMM**	9:00 - 11:00 **CSB** 9:00 - 11:00 **SMI**		9:00 - 11:00 **FPM** 9:00 - 11:00 **SYM**	9:00 - 11:00 **LIS** 9:00 - 11:00 **NBS**
	11:00 - 12:00 **Cb + Ps**	11:00 - 12:00 **Cb + Ps**		11:00 - 12:00 **Cb + Ps**	11:00 - 12:00 **Cb + Ps**
	12:00 - 13:00 **Rusty Rodriguez**	12:00 - 13:00 **Dorion Sagan**		12:00 - 13:00 **Catherine Masson**	12:00 - 13:00 **Jan Sapp**
	12:00 -14:30 **Lunch**	12:00 -14:30 **Lunch**		12:00 -14:30 **Lunch**	12:00 -14:30 **Lunch**
16:00 - 18:00 **Re - gistration**	14:30 - 16:30 **CSG** 14:30 - 16:30 **EMM**	14:30 - 16:30 **EFM** 14:30 - 16:30 **SMI**	14:30 - 18:30 **STR Working Group**	14:30 - 15:30 **FPM** 14:30 - 15:30 **SYM** 15:30 - 16:30 **IAM** 15:30 - 16:30 **HOL**	14:30 - 16:30 **LIS** 14:30 - 16:30 **MWH** 14:30 - 16:30 **REB**
	16:30 - 17:15 **Cb + Ps**	16:30 - 17:15 **Cb + Ps**		16:30 - 17:15 **Cb + Ps**	16:30 - 17:15 **Cb + TSY**
18:00 - 19:30 **Welcome reception**	17:15 - 19:00 **EBC** 17:15 - 18:30 **EMM**	17:15 - 18:30 **EFM** 17:15 - 18:30 **SBI**		17:15 - 19:00 **IAM** 17:15 - 19:00 **HOL**	17:15 - 19:00 **TSY** 17:15 - 19:00 **CHA** 17:15 - 19:00 **REB**
		20:00 **Conference Dinner**		19:00 - 20:00 **Wine of the World**	19:00 - 19:30 **Closing Session**

CHA = Connecting habitats
CSB = Communication, symbiosis and behaviour
CSG = Chemosynthetic symbioses in the genomic and post-genomic era
EBC = Endophytism and biological control
EFM = Ecology and functionality of microbiomes (plants, vertebrates, invertebrates)
EMM = Establishment and maintenance of mutualism
FPM = Functional plant microbiomes: effects of endophytes and epiphytes on plants
HOL = Holobionts as players in ecological stress gradients
IAM = Interdisciplinary approach to mycorrhizal symbiosis
LIS = Lichen symbionts: marriage, divorce, and domestic partnership
MWH = Molecular and cell biology of *Wolbachia*-host interaction
NBS = Nematode-bacteria symbioses
REB = Reticulate evolution before and after the modern synthesis: historical and epistemological perspectives and wider applications beyond traditional fields
SBI = Symbiont-based improvement of plant nutraceutical value
SLS = Symbiotic lifestyle switching
SMI = Symbionts in multipartite interactions: ecology, evolution and regulatory mechanisms
STR = Strigolactones role in plant symbiosis in the rhizosphere
SYM = Symbiotic microbes - new frontiers in applied biotechnology
TSY = Teaching symbiosis: look at successful strategies

Room 6.1.36	**Room 3.2.14**
Coffee breack + Poster session Cb + Ps Poster Hall	**Room 2.2.15**

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Timetable

Monday 13th - Room 3.2.14

8:30-9:00 Opening session

Monday 13th - Room 3.2.14

Establishment and maintenance of mutualism (EMM)

Martin Kaltenpoth (Max Planck Institute for Chemical Ecology, Germany)

Oral presentations

9:00-9:05 Martin Kaltenpoth, Max Planck Institute for Chemical Ecology, Germany
"Establishment and maintenance of mutualism - introduction"
9:05-9:45 Abdelaziz Heddi (Keynote speaker), University of Lyon, INRA, France
"Insights into evolutionary and immune processes paralleling endosymbiosis establishment and maintenance"
9:45-10:00 Joan Strassmann, Washington University, USA
"Interactions between the social amoeba *Dictyostelium discoideum* and soil bacteria"
10:00-10:15 John Brooks, Northwestern University Feinberg School of Medicine, USA
"A sensor histidine kinase regulates biofilm formation, motility, and cyclic-di-GMP in the symbiont, *Vibrio fischeri*"
10:15-10:30 Lisl Esherick, Stanford University School of Medicine, USA
"Possible role of C-type lectins in the establishment of cnidarian-dinoflagellate symbiosis"
10:30-10:45 Filip Husnik, University of Montana, USA
"On the origin and evolution of a tripartite nested mealybug symbiosis"
10:45-11:00 Erik Hom, University of Mississippi, USA
"Of chance and necessity: a new suite of fungal-algal mutualisms created via synthetic ecology"

11:00-12:00 Coffee break + Poster session
12:00-13:00 Plenary lecture – Rusty Rodriguez
13:00-14:30 Lunch

14:30-15:00 David Queller, Washington University in St. Louis, USA
"General models of the evolution of interspecific interactions"
15:00-15:15 Florent Masson, INSA de Lyon, France
"Dual function of antimicrobial peptides in insect endosymbiosis: does the bacteriome immunity have a 'split personality'?"
15:15-15:30 Tobias Engl, Max Planck Institute for Chemical Ecology, Germany
"Are beewolf symbionts fighting a losing battle against the radical host protection?"
15:30-15:45 Yoshitomo Kikuchi, National Institute of Advanced Industrial Science and Technology, Japan
"Symbiont mechanisms for stabilizing an insect-microbe symbiosis with horizontal transmission"
15:45-16:00 Clinton Oakley, Victoria University of Wellington, New Zeland
"The effects of symbiotic state on the proteome of the model cnidarian *Aiptasia pulchella*"
16:00-16:15 Sophie Colston, University of Connecticut, USA
"Identification of a host-specific locus found in the digestive tract microbiota of medicinal leeches"
16.15-16:30 Virginia Weis, Oregon State University, USA
"Role of innate immunity in the regulation of cnidarian-dinoflagellate symbioses"

16:30-17:15 Coffee break + Poster session

17:15-17:30 Mary Beth Saffo, University of Rhode Island, USA
"Genomic complexities in evolution of the beneficial apicomplexan *Nephromyces* from parasitic ancestors"
17:30-17:45 Spencer Nyholm, University of Connecticut, USA
"Hemocyte response in the symbiosis between the Hawaiian Bobtail Squid, *Euprymna scolopes*, and the bioluminescent bacterium, *Vibrio fischeri*"
17:45-18:00 Tsubasa Ohbayashi, Hokkaido University, Japan
"The role of crypt-specific cysteine-rich proteins (CCRs) in the bean bug-*Burkholderia* symbiosis"
18:00-18:15 Jon Seal, University of Texas at Tyler, USA
"Stability and instability of host-symbiont combinations among higher fungus-gardening ants"
18:15-18:30 Simon Davy, Victoria University of Wellington, New Zealand
"Symbiont diversity and its influence on host metabolite profile in a model cnidarian-dinoflagellate symbiosis"

Poster presentations

P1. Agathe Lecointe, Laboratory for Biological Geochemistry, EPFL, Switzerland
"Highly dynamic host regulation of *Symbiodinium* population during the ontogeny of the symbiotic scleractinian coral"
P2. Cecile Sabourault, University of Nice, France
"Symbiosome membranes characterization, or how to decipher molecular interactions between cnidarians and their dinoflagellate symbionts"
P3. Elfie Stocker-Wörgötter, University of Salzburg, Austria
"Ancestral plant forms conquering terrestrial habitats: survival, biodiversity, evolution of symbiotic lichenized and freeliving *Trentepohliales*"
P4. Fabrice Not, Station Biologique de Roscoff, France
"Metabolomic of mutualism establishment in planktonic photosymbiosis"
P5. Maria Cristina Motta, Instituto de Biofísica Carlos Chagas Filho, Brazil
"Endosymbiosis in trypanosomatid protozoa: the bacterium division is controlled during the host cell cycle"
P6. Mariya Zhukova, University of Copenhagen, Denmark
"Transmission of dominant *Wolbachia* and *Mollicutes* gut bacteria in leaf-cutter ants"
P7. Mélisandre Téfit, Ecole Normale Supérieure de Lyon, France
"Growing faster or growing too fast? Effects of *Lactobacillus plantarum* on *Drosophila* fitness"
P8. Patrícia Ventura, Université Nice Sophia-Antipolis
"Short-term and long-term acclimation to ocean acidification of symbiotic cnidarian *Anemonia viridis*"
P9. Pu Yang, University of Groningen, The Netherlands
"The type 3 secretion system (T3SS) improves bacterial fitness in the mycosphere"
P10. Sheila Kitchens, Oregon State University, USA
"Sphingolipids in cnidarian-dinoflagellate interactions: investigating the role of the sphingosine rheostat during symbiont colonization"
P11. Vincent Dani, University of Nice Sophia-Antipolis, France
"NPC proteins are key players in molecular interactions between cnidarians and their dinoflagellate endosymbionts"

Timetable

Monday 13th - Room 6.1.36

Symbiotic lifestyle switching (SLS)

Russell J. Rodriguez (Adaptive Symbiotic Technologies, USA)

Stanley Freeman (The Volcani Center, Israel)

Oral presentations

9:00-9:15 Stanley Freeman (Keynote speaker), The Volcani Center, Israel
"Symbiotic lifestyles between and among three fungal species with the ambrosia beetle *Euwallacea* nr. f*ornicatus* and different host plants"
9:15-9:30 Regina S. Redman (keynote speaker), Adaptive Symbiotic Technologies
"The role of genetic and environmental landscapes on plant-fungal symbiosis"
9:30-9:45 Thomas Wolpert (keynote speaker), Oregon State University, USA
"Victoria blight and pathogen exploitation of plant defense genes"
9:45-10:00 James White (keynote speaker), Rutgers University, USA
"The proposed role of virulence-suppressive compounds in maintenance of latency in cranberry fruit rot disease and its relevance to endophytism"
10:00-10:15 Ana Corrêa, Universidade de Lisboa, Portugal
"Living together, behaving as one: rethinking mycorrhizal systems"
10:15-10:30 Anna Zaidman-Rémy, INSA de Lyon, France
"How do insects optimize their symbiotic investment? Endosymbiont control and load adjustment to insect physiological needs"
10:30-10:45 Zerrin Uzum, Institute for Natural Product Research and Infection Biology, Germany
"Active invasion of bacteria into living fungal cells"
10:45-11:00 Laura Runyen-Janecky, University of Richmond, USA
"Characterization of *Sodalis glossinidius* heme iron acquisition and homeostasis genes"

Poster presentation

P12. Francisco Cerqueira, Universidade de Lisboa, Portugal
"Symbiosis establishment in the rhizosphere: specific tasks for specific partners"

Timetable

Monday 13th - Room 6.1.36

Chemosynthetic symbioses in the genomic and post-genomic era (CSG)

François Lallier (UPMC-Univ Paris 6, France)

Jillian Petersen (Max Planck Institute, Germany)

Oral presentations

14:30-14:50 Jillian Petersen (Keynote speaker), Max Planck Institute for Marine Microbiology, Germany
"The power of 'omics' for revealing hidden metabolic potential and cryptic host-symbiont interactions in chemosynthetic symbioses"
14:50-15:07 Arnaud Tanguy (Keynote speaker), Station Biologique de Roscoff, France
"Host-symbionts relationship in the deep-sea mussel *Bathymodiolus* spp explored with transcriptomic approaches: a state of the art"
15:07-15:24 Sebastien Duperron, Sorbonne Universités, Université Pierre et Marie Curie, France
"Revisiting the flexible symbioses of deep-sea mussels using NGS, FISH and live experiments"
15.24-15:41 Satoshi Nakagawa (keynote speaker), Kyoto University,Japan
"Genomic and population genetic analysis of gastropod symbionts in deep-sea hydrothermal fields"
15:41-15:58 Stephanie Markert (keynote speaker), Institute of Marine Biotechnology, Germany
"Marine chemoautotrophic symbioses examined by physiological proteomics"
15:58-16:15 Camille Détrée, Station Biologique de Roscoff, France
"Using proteomics to better understand a symbiosis in deep sea hydrothermal vent"
16:15-16:30 Marie-Anne Cambon-Bonavita, Laboratory of Microbiology of Extreme Environments, France
"*Rimicaris exoculata*: toward better understandings of a complex symbiosis"

Poster presentations

P13. Aurélie Tasiemski, Université de Lille, France
"Phenotypic convergence and conservation of immune processes involved in the establishment of detoxifying symbiosis between coastal and hydrothermal annelids"
P14. François H. Lallier, Station Biologique de Roscoff, France
"Apoptosis and symbiosis in bivalve mollusks"
P15. Kamil M. Szafranski, Université Paris-Sud, France
"Tracking the life-cycles of deep-sea metazoan's bacterial symbionts at hydrothermal vents and cold seeps"
P16. Mary Jean, Station Biologique de Roscoff, France
"The peptidoglycan recognition protein, a potential candidate for the regulation of the chemosynthetic symbiosis in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus*"
P17. Simon Le Bloa, Laboratoire de Microbiologie des Environnements Extrêmes
"Toward a better understanding of the symbiotic relationships in *Rimicaris exoculata* model"
P18. Tjorven Hinzke, Institute of Marine Biotechnology, Germany
"Metabolic variability between symbiont subpopulations in *Riftia pachyptila*"

Monday 13th - Room 6.1.36

Endophytism and Biological Control (EBC)

Luis Vicente Lopez-Llorca (Universidad de Alicante, Spain)

Oral presentations

17:15-17:35 Luis Vicente Lopez-Llorca (Keynote speaker), University of Alicante, Spain
“Interactomics, plant growth promotion and biocontrol”
17:35-17:50 Elizabeth Czerwinski, Indiana University, USA
“*Azospirillum brasilense* beyond the rhizosphere”
17:50-18:10 Ernesto Alejandro Zavala-González (Keynote speaker), University of Alicante, Spain
“Effects of the nematophagous fungus *Pochonia chlamydosporia* on the development of tomato and *Arabidopsis*”
18:10-18:25 Naveen Kumar Arora, BBA University, India
“Fluorescent pseudomonads as efficient plant growth promoting and biocontrol agents for diverse crops under saline conditions”
18:25-18:45 Nuria Escudero (Keynote speaker), University of Alicante, Spain
“Analysis of the tritrophic interaction: Tomato, *Meloidogyne javanica* and *Pochonia chlamydosporia*”
18:45-19:00 Marta Alves, University of Aveiro, Portugal
“Bacterial community associated to *Monochamus*, the insect-vector of pine wilt disease”

Poster presentations

P19. James White, Rutgers University, USA
“A bacterial endophyte that enhances heat stress tolerance and insect feeding deterrence in ornamental *Hosta* cultivar”
P20. Kumkum Azad, University of Saskatchewan, Canada
“Fungal endophytes: a strategy for mitigating salt and drought stress on plant growth”
P21. Maria Clara Vieira dos Santos, University of Coimbra, Portugal
“A novel biocontrol strategy against root-knot nematodes - exploring the interaction *Pochonia chlamydosporia*-plant defence mechanisms”

Tuesday 14th - Room 3.2.14

Symbionts in multipartite interactions: ecology, evolution and regulatory mechanisms (SMI)

Flore Zélé (Universidade de Lisboa, Portugal)

Sara Magalhães (Universidade de Lisboa, Portugal)

Maria J.Pozo (Consejo Superior de Investigaciones Científicas, Spain)

Oral presentations

9:00-9:30 Christoph Vorburger (Keynote speaker), ETH Zürich & Eawag, Switzerland
“Endosymbionts mediate host-parasitoid coevolution and alter parasitoid community composition”
9:30-9:50 Enric Frago, Wageningen University, The Netherlands
“Insect symbionts mediate indirect interactions in aphid communities”
9:50-10:10 Ailsa McLean, University of Oxford, UK
“Symbionts protecting against multiple natural enemies”
10:10-10:30 Melanie Smee, University of York, UK
“Choose your partner carefully: variation in aphid phenotypes when multiple bacterial symbionts coexist”
10:30-10:50 Melissa Whitaker, Harvard University, USA
“Do symbiotic gut bacteria influence interactions between ants and lycaenid butterflies?”
10:50-11:00 Maria J. Pozo, Consejo Superior de Investigaciones Científicas, Spain
“Using three-way interactions between plants, microbes and arthropods to enhance crop protection and production”

11:00-12:00 Coffee break + Poster session
12:00-13:00 Plenary lecture – Dorion Sagan
13:00-14:30 Lunch

14:30-15:00 Marcia González-Teuber (Keynote speaker), Universidad de La Serena, Chile
“Mutualistic ants as an indirect defence against leaf pathogens”
15:00-15:20 Alejandro Manzano-Marín, University of Valencia, Spain
“No subfamily is perfect…: Secondary symbiont settlement, replacement and internalization in the subfamily Lachninae of aphids”
15:20-15:40 Sven Gould, University of Dusseldorf, Germany
“Green sea slugs: plastid symbiosis in animal cells”
15:40-16:00 Frederique Guinel, Wilfrid Laurier University, Canada
“High endogenous levels of cytokinins differently affect the entry of beneficial root symbionts in the pea nodulation mutant E151”
16:00-16:20 Liu Yin-Quan, Zhejiang University, China
“Differential responses of the whitefly *Bemisia tabaci* symbionts to unfavorable low and high temperatures”
16:20-16:40 Sónia Duarte, LNEC, Portugal
“Effects of starvation in *Reticulitermes grassei* symbiotic flagellate protists community”

Poster presentations

P22. Cessa Rauch, Heinrich-Heine-University, Germany
"Starvation response in the plastid-bearing sea slug *Elysia viridis*"
P23. Elad Chiel, University of Haifa-Oranim, Israel
"Can bacterial symbionts push galling aphid over the speciation cliff?"
P24. Eleanor Heyworth, University of York, UK
"Horizontal transfer leads to competition and loss in an unstable pea aphid multiple endosymbiont infection"
P25. Girish Beedessee, Okinawa Institute of Science and Technology Graduate University, Japan
"Genome-wide survey of polyketide synthases
P26. Marco A. Villanueva, , Instituto de Ciencias del Mar y Limnología-UNAM, México
"Molecular features of the receptor for activated C kinase from *Symbiodinium microadriaticum* ssp. *microadriaticum*, SmicRACK1"
P27. Polina Dgebuadze, A.N. Severtson Institute of Ecology and Evolution, Russia
"Host preference of symbiotic gastropods (Eulimidae): experimental data"
P28. Sebastian Baumgarten, King Abdullah University of Science and Technology, Saudi Arabia
"The genome of *Aiptasia*, a sea anemone model for coral biology"
in the symbiotic dinoflagellate *Symbiodinium minutum*"
P29. Thomas Ogao Onchuru, Max Planck Institute for Chemical Ecology, Germany
"The good, the bad, and their regulation: symbiosis, parasitism, and immune response in an insect gut"

Tuesday 14th - Room 6.1.36

Communication, symbiosis and behaviour (CSB)

Francisco Dionisio and Luis Carvalho (Universidade de Lisboa, Portugal)

Oral presentations

9:00-9:40 Karina Xavier (Keynote speaker), Instituto Gulbenkian de Ciência, Portugal
"Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota"
9:40-10:00 Debra Brock, Washington University in Saint Louis, USA
"Cooperative interactions with bacteria aid in toxin resistance in the social amoeba *Dictyostelium discoideum*"
10:00-10:20 Jacques Batut, INRA, France
"Sophisticated communication controls root infection in the rhizobium-legume symbiosis"
10:20-10:40 Louis Tisa, University of New Hampshire, USA
"*Frankia* Genomics and Genome-guided approaches toward understanding the actinorhizal symbiosis"
10:40-11:00 Aurélie Tasiemski, Université de Lille, France
"The complementary production of antibiotics by the leech *Hirudo verbana* and its gut symbiont *Aeromonas veronii* reinforces their mutualistic association"

Poster presentations

P30. Alessandra Pepe, University of Pisa, Italy
"Self-recognition and incompatibility affect the structure and interconnectedness of mycorrhizal networks formed by different isolates within Glomeraceae"
P31. Irshad Ul Haq, University of Groningen, The Netherlands
"Transcriptional landscape of *Burkholderia* terrae BS001 populations upon confrontation with the soil fungus *Lyophyllum* sp. strain Karsten"
P32. Johana Revel, University of Nice-Sophia-Antipolis, France
"The molecular communication in cnidarian-dinoflagellate symbiosis"
P33. Temir Britayev, Institute of Ecology and Evolution RAS, Russia
"Establishment of symbiotic assemblage associated with polcilloporid coral: two years planting experiment"

Timetable

Tuesday 14th - Room 6.1.36

Ecology and functionality of microbiomes (plants, vertebrates, invertebrates) (IFM)

J. Dick van Elsas (University of Groningen, Netherlands)

Oral presentations

14:30-15:00 J.Dick van Elsas (Keynote speaker), University of Groningen, The Netherlands
“Mechanisms in microbiomes-bacterial-fungal interactions”
15:00-15:20 Martin Kaltenpoth, Max Planck Institute for Chemical Ecology, Germany
“The hitchhiker’s guide to symbiosis: A plant pathogen as an insect’s defensive mutualist”
15:20-15:40 Maryam Chaib de Mares, University of Groningen, The Netherlands
“Microbial community structure and functional attributes of symbionts associated to sponges of the genera *Aplysina* and *Dysidea*”
15:40-16:00 Luis Teixeira, Instituto Gulbenkian de Ciência, Portugal
“Identification of stable and beneficial *Drosophila melanogaster* gut microbiota”
16:00-16:20 Luis M. Bolaños, Universidad Nacional Autónoma de México, Mexico
“Species-specific diversity of novel bacterial lineages and differential abundance of pathways for toxic compound degradation in scorpion gut microbiota”

16:30-17:15 Coffee break + Poster session

17:15-17:45 Joana F. Salles (keynote speaker), University of Groningen, The Netherlands
“The microbiome of bird eggs”
17:45-18:00 Sergios-Orestis Kolokotronis, Fordham University, USA
“Bacterial communities of blacklegged ticks (*Ixodes scapularis*) across developmental stages”
18:00-18:15 Mariana Reyes-Prieto, University of Valencia, Spain
“SGDB: Symbiotic genomes database for the integration and access to knowledge on host-symbiont relationships”
18:15-18:30 Fredrick Lee, Indiana University, USA
“The honey bee gut microbiome is involved in the breakdown of host dietary macromolecules”

Poster presentations

P34. Ana Regueiras, University of Porto, Portugal
“The microbial community within the marine sponge *Hymeniacidon perlevis*: diversity and changes under laboratory conditions”
P35. Ian Probert, Station Biologique de Roscoff, France
“Photosynthetic symbionts of planktonic *Radiolaria*”
P36. Juliana Melo, Universidade de Lisboa, Portugal
“The cultivation system can influence the physiological functionality and social interaction of phosphate solubilizing bacteria from the rhizosphere of *Carica papaya* L.!”
P37. Katrin Kellner, University of Texas at Tyler, USA
“Bacterial community composition and diversity in an ancestral ant fungus symbiosis”
P38. Manuela Giovannetti, University of Pisa, Italy
“Multitrophic interactions in the sporosphere of the plant beneficial symbiont *Rhizoglomus intraradices*”
P39. Teresa Lino-Neto, University of Minho, Portugal
“Mycorrhizal community of cork oak (*Quercus suber* L.) forests in two different agro-forestry ecosystems”
P40. Ana Soares, Universidade de Lisboa, Portugal
“Is the output of the plant mycorrhizal symbiosis determined by the plant and fungal symbionts, or by the microbiome?”
P41. James White, Rutgers University, USA
“Do *Bacillus* endophytes and epiphytes of food plants colonize the human digestive tract?”

Timetable

Tuesday 14th - Room 3.2.14

Symbiont-based improvement of plant nutraceutical value (SBI)

Manuela Giovannetti (Università di Pisa, Italy)

Oral presentations

17:15-17:25 Berta Graziella (Keynote speaker), Università del Piemonte Orientale, Italy
“Arbuscular mycorrhizal fungi and rhizobacteria for the improvement of the nutraceutical values of crops”
17:25-17:35 Cristiana Sbrana (Keynote speaker), CNR, Italy
“Health-promoting phytochemicals in artichoke and tomato as affected by beneficial mycorrhizal symbionts”
17:35-17:45 Thomas Fester (Keynote speaker), Helmholtz-Centre, Germany
“Apocarotenoids from arbuscular mycorrhizal roots - persistent mysteries”
17:45-17:55 Szymon Zubek (Keynote speaker), Jagiellonian University, Poland
“Interactions of medicinal plants with arbuscular mycorrhizal fungi”
17:55-18:05 Marco Cosme (Keynote speaker), Freie Universität Berlin, Germany
“Impact of mycorrhiza on health- and flavour-related food quality in tomato and moringa”
18:05-18:30 General discussion

Thursday 16th - Room 3.2.14

Symbiotic microbes - new frontiers in applied biotechnology (SYM)

Katarzyna Turnau (Jagiellonian University, Poland)

Cristina Prandi (Università di Torino, Italy)

Oral presentations

9:00-9:15 Katarzyna Turnau, Jagiellonian University, Poland, and Cristina Prandi, Università di Torino, Italy
“Symbiotic microbes - new frontiers in applied biotechnology”
9:15-9:35 Hinanit Koltai (Keynote speaker), The Volcani Center, Israel
“Potential practical implementation of strigolactones for plant interactions”
9:35-9:55 Yoram Kapulnik (Keynote speaker), The Volcani Center, Israel
“Application of Mycorrhiza symbiosis- present and future prospects”
9:55-10:10 Andrea Genre, Università di Torino, Italy
“Presymbiotic signaling in arbuscular mycorrhizas”
10:10-10:25 Michael H. Walter, Leibniz-Institute of Plant Biochemistry, Germany
“Mycorrhization for combating parasitic weeds: approaches to control strigolactone germination stimulant exudation”
10:25-10:35 Rafał Ważny, Jagiellonian University, Poland
“Inoculation of *Lactuca serriola* with endophytic and mycorrhizal fungi improves phytoremediation”
10:35-10:45 Piotr Rozpądek, Jagiellonian University, Poland
“Strigolactone deficient *Arabidopsis thaliana* mutants max1 and max4 are hindered in their ability to form a symbiotic association with the endophytic fungus *Mucor plumbeus*”
10:45-11:00 Allison H. Kerwin, University of Connecticut, USA
“Functional characterization of the bacterial community associated with a reproductive gland of the Hawaiian bobtail squid, *Euprymna scolopes*”

11:00-12:00 Coffee break + Poster session
12:00-13:00 Plenary lecture – Catherine Masson
13:00-14:30 Lunch

14:30-14:45 Ángela Figás Segura, Universitat de València, Spain
“Exploring the supply and recycling of nutrients of *Ramalina farinacea*-associated culturable bacteria: contribution to the lichen symbiosis and biotechnology”
14:45-15:00 Olivier Detournay, Coral Guardian, France
“Functional characterization of the TGFbeta pathway during the onset of symbiosis in a scleractinian model”
15:00-15:30 General discussion

Poster presentations

P42. Janusz Blaszkowski, University of Technology in Szczecin, Poland
“Why the recognition of the identity of arbuscular mycorrhizal fungi (Glomeromycota) is essential to know and practically use their symbiotic lifestyle”
P43. Simone Belmondo, University of Torino, Italy
“Functional genomics tools to investigate fungal responses to strigolactones”
P44. Susana Castro-Sowinski, IIBCE, Uruguay
“Draft genome of the plant-growth promoting and Cr(VI)-reducing bacterium *Delftia* sp. JD2”
P45. Susana Castro-Sowinski, IIBCE, Uruguay
“The microbiome of the Antarctic oligochaeta *Grania* sp.: occurrence of hydrolytic enzyme-producing microorganisms”
P46. Theo Ruissen, Norwegian Institute for Agricultural and Environmental Research, Norway
“Specificity in plant - arbuscular mycorrhizal fungal (AMF) interactions; reality or a *fata morgana*?”

Thursday 16th - Room 6.1.36

Functional plant microbiomes: effects of endophytes and epiphytes on plants (FPM)

James White (Rutgers University, USA)

Charles Bacon (Russell Research Center, USA)

Russell J. Rodriguez (Adaptive Symbiotic Technologies, USA)

Oral presentations

9:00-9:20 James White (Keynote speaker), Rutgers University, USA
“Functions and mechanisms of plant microbiomes”
9:20-9:40 Charles Bacon (Keynote speaker), Russell Research Center, USA
“Endophytic microbiomes: defensive functions and responsible metabolites of microbial endophytes”
9:40-10:00 Rusty Rodriguez (Keynote speaker), Adaptive Symbiotic Technologies, USA
“Symbiotic adaptation: using endophytes to reprogram plant physiology for agricultural sustainability and food security”
10:00-10:20 Carolyn A. Young (Keynote speaker), The Samuel Roberts Noble Foundation, USA
“Discovery, diversity and utilization of endophytes: *Epichloë* species in wild and forage grasses”
10:20-10:40 Miguel J. Beltran-Garcia (Keynote speaker), Universidad Autonoma de Guadalajara, Mexico
“When you drink Tequila next time, think in endophytes: cultivable bacteria of *Agave tequilana* and the organic nitrogen transfer”

10:40-11:00 Anthony Glenn (Keynote speaker), Russell Research Center, USA
“Thank you, may I have another cluster? Horizontal transfer of xenobiotic metabolizing gene clusters among fungal endophytes of maize”

11:00-12:00 Coffee break + Poster session
12:00-13:00 Plenary lecture – Catherine Masson
13:00-14:30 Lunch

14:30-14:50 Florian Ulm, Universidade de Lisboa, Portugal
“In situ differential response of soil, roots and mycorrhizosphere to altered N/P constraints. Evidence from an N-manipulation experiment in a Mediterranean ecosystem”
14:50-15:10 Sevda Haghi Kia, Goethe University, Germany
“Effect of biotic and abiotic variables on the interaction between plants and fungal endophytes”
15:10-15:30 Zakia Boubakir, University of Saskatchewan, Canada
“Using *Trichoderma harzianum* strain TSTh20-1 for revegetation and bio-remediation of oil-contaminated soil”

Poster presentations

P47. Akhtemova Gulnar, All-Russia Research Institute for Agricultural Microbiology, Russia
“Culturable endophytic bacteria of pea (*Pisum sativum* L.)”
P48. Febri Doni, Universiti Kebangsaan, Malaysia
“Transformational approaches in paddy planting: the case of *Trichoderma*-paddy interactions in a biocide-free ecosystem”
P49. Jan de Vries, Heinrich-Heine-Universitaet Duesseldorf, Germany
“Phytohormone signalling and the *Nostoc-Azolla* symbiosis”
P50. Marzena Sujkowska-Rybkowska, Warsaw University of Life Sciences, Poland
“Localization of calcium ions in mycorrhizal roots and root nodules of *Medicago truncatula* in response to aluminum stress”

Thursday 16th - Room 3.2.14

Holobionts as players in ecological stress gradients

Karin Pritsch (Helmholtz Zentrum Muenchen, Germany)

Thorsten Grams (Technische Universitaet Muenchen, Germany)

Oral presentations

15:30-15:40 Karin Pritsch, Helmholtz Zentrum Muenchen, Germany and Thorsten Grams, Technische Universitaet Muenchen, Germany
“Holobionts as players in ecological stress gradients – Introduction to the session”
15:40-16:10 Marc-André Selosse (keynote speaker), Sorbonne Universités, France
“The evolution of interdependency by neutral evolution in holobionts”
16:10-16:30 Viviane Radl, Helmholtz Zentrum Munchen, Germany
“*Microvirga vignae*: a novel legume symbiont adapted to semi-arid soils?”

16:30-17:00 Coffee break

17:00-17:20 Short presentations of posters

17:20-17:40 Daniel Martin, Centre d'Estudis Avançats de Blanes, Spain
"Comparative phylogeography of two symbiotic dorvilleid polychaetes with contrasting host-crab and bathymetric patterns"
17:40-18:00 Thomas Krueger, École Polytechnique Fédérale de Lausanne, Switzerland
"Autotrophic and heterotrophic nutrient fluxes in the dinoflagellate-coral symbiosis – A NanoSIMS perspective"
18:00-18:20 Laura Leite, Universidade de Aveiro, Portugal
"Potential effect of metal contamination on the microbiome of Manila clam (*Venerupis phillipinarum*): a culture-based approach"
18:20-18:40 Aileen Berasategui López, Max Planck Institute for Chemical Ecology, Germany
"Role of endosymbiotic gut bacteria in the detoxification terpenes by the pine weevil (*Hylobius abietis*)"
18:40-19:00 Yoav Soen, Weizmann Institute of Science, Israel
"Bridging ecology and evolution by symbiosis and epigenesis"

Poster presentations

P51. Andres Gutierrez, Station Biologique de Roscoff, France
"Concentration and S-isotopic composition of dimethyl sulfur compounds in symbiotic radiolaria: a potentially important unaccounted source for marine DMS"
P53. Daniel Martin, Centre d'Estudis Avançats de Blanes, Spain
"1998 - 2014: updating the quantification of symbiotic polychaetes and their hosts"
P54. Sergio Enrico Favero-Longo, University of Torino, Italy
"The microbiome of *Peltigera ponojensis* Gyeln"

Thursday 16th - Room 6.1.36

Interdisciplinary approach to mycorrhizal symbiosis (IAM)

Manju M. Gupta (University of Delhi, India)

Oral presentations

15:30-16:00 Christophe Roux (keynote speaker), Université de Toulouse, France
"The lifestyle of arbuscular mycorrhizal fungi in the light of genomics"
16:00-16:30 Jacqueline Baar (keynote speaker), Biomygreen BV, Velp, The Netherland
"A custom fit approach for (cost-)effective use of mycorrhizal fungi"
17:00-17:30 Manju M. Gupta (keynote speaker), University of Delhi, India
"Bioinformatics tools and computer applications for developing insights to understanding arbuscular mycorrhizal symbiosis in India"
17:30-17:50 Mahaveer P. Sharma, Indian Council of Agricultural Research- Directorate of Soybean Research, India
"Contribution of native AM fungi to soil carbon sequestration assessed in the form of glomalin and C-stocks in different soil and crop management practices of soybean-based cropping system"
17:50-18:10 Oksana Y. Shtark, All-Russia Research Institute for Agricultural Microbiology, Russia
"Comprehensive analysis of pea (*Pisum sativum* L.) mutants with defects in both arbuscular mycorrhiza and nodule development"
18:10-18:30 Veena Pande, Kumaun University, India
"Molecular characterization and genetic diversity analysis of ectomycorrhizal fungi associated with *Quercus leucotrichophora* (Oak)"
18:30-19:00 General discussion

Poster presentations

P55. Amornrat Chaiyasen, Chiang Mai University, Thailand
"Arbuscular mycorrhizal communities analysis and their effect on growth of *Aquilaria crassna* and *Tectona grandis*"
P56. Inês Rocha, University of Coimbra, Portugal
"Application of arbuscular mycorrhizal fungi inoculum through seed coating for large scale agriculture"
P57. Jaturong Kumla, Chiang Mai University, Thailand
"The ectomycorrhizal status determination of *Phlebopus portentosus* using isotopic analysis"
P58. Katherine Muller, University of Minnesota, USA
"Measuring the benefits of symbiotic rhizobia to legume hosts: Importance of timing, efficiency, and resource hoarding in single- and multi-strain inoculation"
P59. M. Lourdes Adriano-Anaya, Universidad Autónoma de Chiapas
"Differential effect of abiotic factors on mycorrhization of *Catharanthus roseus* (L.) G. Don in soil of Chiapas, Mexico"
P60. Manju Gupta, University of Delhi, India
"Resolving phylogeny of Indian Glomeromycota"
P61. Rui S. Oliveira, University of Coimbra, Portugal
"Seed coating with plant growth promoting microorganisms as an ecotechnological approach for sustainable agriculture"
P62. Saisamorn Lumyong, Chiang Mai University, Thailand
"Cultural characterization of *Pisolithus* species collected from northern Thailand and their *in vitro* mycorrhization with *Eucalyptus camaldulensis* seedling"
P63. Uchinomiya Kouki, Kyushu University, Japan
"Mathematical model for optimal resource allocation in the plant mycorrhizal symbiosis"

Friday 17th - Room 3.2.14

Nematode-bacteria symbioses (NBS)

Patricia Stock (University of Arizona, USA)

Oral presentations

9:00-9:20 Silvia Bulgheresi (keynote speaker), University of Vienna, Austria
"Dressed to cooperate: understanding symbiont spatial order on the host surface"
9:20-9:40 Patricia Stock (keynote speaker), University of Arizona, USA
"The epic friendship of *Steinernema* nematodes and their bacterial symbionts"
9:40-10:00 Paula V. Morais (keynote speaker), University of Coimbra, Portugal
"Bacteria associated with *Bursaphelenchus xylophilus*: a role in Pine Wilt Disease or a positive role for the plant?"
10:00-10:20 Mark Blaxter (keynote speaker), University of Edinburgh, UK
"Genomic signatures of coevolution in living and fossil *Wolbachia*"
10:20-10:40 Barton Slatko (keynote speaker), New England Biolabs, USA
"Targeting the *Wolbachia* endosymbiont for human filarial nematode diseases and other insect-borne pathogens"
10:40-11:00 Silvia Libro, New England Biolabs, USA
"Characterization of immune response genes in the parasitic nematode *Brugia malayi*"

Poster presentations

P64. Diogo Neves Proença, University of Coimbra, Portugal
"Link between *Bursaphelenchus xylophilus*-associated bacteria and endophytic microbial community of *Pinus* with Pine Wilt disease"
P65. Jessica Morera, Universidad de Costa Rica, Costa Rica
"Ultrastructural, molecular and phylogenetic characterization of nematodes in the *Cecropia-Azteca* symbiosis, with emphasis on Sclerorhabditis sp. (Nematoda: Rhabditidae)"
P66. John G. McMullen II, University of Arizona, USA
"Role of T6SS locus in *Xenorhabdus bovienii* (Gamma-*Proteobacteria*), the bacterial symbionts of *Steinernema* nematodes"
P67. Patricia Stock, University of Arizona, USA
"Crude extracts of the entomopathogenic bacterium, *Photorhabdus l. sonorensis* (Enterobacteriaceae) and their effect on two plant parasitic nematodes: *Meloidogyne incognita* and *Anguina pacificae* (Nematoda: Tylenchina)"
P68. Wieslaw J. Kozek, University of Puerto Rico, USA
"Electron microscopy, prokaryotic endosymbionts and changing concepts of the paradigms of parasitic associations"

Friday 17th - Room 6.1.36

Lichen symbionts: marriage, divorce, and domestic partnership (LIS)

William B. Sanders (Florida Gulf Coast University, USA)

Elfie Stocker-Wörgötter (University of Graz, Austria)

Oral presentations

9:00-9:40 Lucia Muggia (keynote speaker), University of Trieste, Italy
"Challenging symbiont association patterns in lichens"
9:40-10:00 Ioana Onut Brännström, Uppsala Universitet, Sweden
"The lichen *Thamnolia vermicularis*, a lonely fungus with many green friends"
10:00-10:20 Ulrike Ruprecht, University of Salzburg, Austria
"Diversity, climate related distribution and species specificity of Antarctic lecideoid lichens"
10:20-10:40 Lucie Vančurová, Charles University in Prague, Czech Republic
"Unexpected phycobiont diversity of *Stereocaulon* lichens"
10:40-11:00 Ulla Kaasalainen, University of Göttingen, Germany
"Photobiont mediated guild structure of epiphytic cyanolichens"

11:00-12:00 Coffee break + Poster session
12:00-13:00 Plenary lecture – Jan Sapp
13:00-14:30 Lunch

14:30-14:50 Anna Voytsekhovich, M.G. Kholodny Institute of Botany, Ukraine
"Diversity of algal component in saxicolous lichen communities of Karadag Nature Reserve (Crimea)"
14:50-15:10 Jana Steinová, Institute for Nanomaterials, Advanced Technologies and innovation, Czech Republic
"Diversity of mycobiont-photobiont associations correlate with reproductive strategies in *Cladonia* lichen species"
15:10-15:30 Myriam Catalá, Universidad Rey Juan Carlos, Spain
"*Ramalina farinacea* symbionts: marriage, polygamy and predominance"
15:30-15:50 Patricia Moya, Instituto ICBIBE, Spain
"Lichens as micro-ecosystems: novel approaches to efficiently reveal the hidden diversity of phycobionts in a single thallus"
15:50-16:10 Eva Barreno, Instituto ICBIBE, Spain
"Ontogeny of cyanobacterial cephalodia and non-photosynthetic symbiotic bacteria in the lichen *Lobaria virens* (Whith.) J.R. Laundon"
16:10-16:30 Annette Brandt, Heinrich-Heine University, Germany
"The potential of resistance of the lichen symbiosis to extraterrestrial conditions: exposure to space and Mars-analogue conditions on the ISS and exposure to simulated Galactic Cosmic Rays"

Poster presentations

P69. Andreas Beck, Botanische Staatssammlung Muenchen, Germany
"Metabolic profiling of Alpine and Ecuadorian lichens"
P70. Andres Sadowsky, Heinrich Heine University, Germany
"Transcriptomics of desiccation tolerance in Antarctic lichen photobionts"
P71. Anna Voytsekhovich, M.G. Kholodny Institute of Botany, Ukraine
"Optionally lichenized fungi of *Hyphodontia* (Agaricomycetes, Schizoporaceae)"
P72. Anna Voytsekhovich, M.G. Kholodny Institute of Botany, Ukraine
"The impact of multiple photobionts on lichen ecology and distribution - an ecophysiological approach"
P73. Eva Barreno, Instituto ICBIBE, Spain
"Phylogenetic analysis of symbiotic *Trebouxia* microalgae found in sorediate lichens of *Parmelia* reveal new monophyletic clades"
P74. Joachim Meeßen, Heinrich-Heine-University, Germany
"The effect of extraterrestrial stressors (UVC- and γ-radiation) on isolated photobionts of the two astrobiological model lichens *Buellia frigida* and *Circinaria gyrosa*"
P75. Lucia Muggia, University of Trieste, Italy
"Water relations in lichens: a case study with the tripartite *Peltigera britannica* and its isolated photobionts"
P76. Lucia Muggia, University of Trieste, Italy
"Rock-inhabiting fungi and their association with algae: where does symbiosis start?"
P77. Ondřej Peksa, The West Bohemian Museum in Pilsen, Czech Republic
"Do saxicolous lichen communities represent ecological guilds assembled on locally adapted photobionts?"
P78. Samantha Fernández Brime, Swedish Museum of Natural History, Sweden
"Exploring the microbiome of the optionally lichenized fungus *Schyzoxylon albescens*"
P79. Silvana Munzi, Universidade de Lisboa, Portugal
"How does *Cladonia portentosa* respond to nitrogen? Effect of form, dose, time of exposure, and PK addition on protein expression"
P80. Tereza Řidká, Charles University in Prague, Czech Republic
"Environmental preferences of photobionts associating with epigeic *Cladonia* communities"
P81. Ulla Kaasalainen, University of Göttingen, Germany
"Reconstruction of fossil lichen communities: from molecules to palaeoecosystems"

Friday 17th - Room 3.2.14

Molecular and cell biology of *Wolbachia*-host interaction (MWH)

Irene L.G. Newton (Indiana University, USA)

Luis Teixeira (Instituto Gulbenkian de Ciência, Portugal)

Oral presentations

14:30-15:00 Wolfgang Miller (keynote spaker), Medical University of Vienna, Austria
"Unorthodox transmission modes of endosymbionts in hybrids and the symbiotic origin of speciation"
15:00-15:25 Irene Newton (keynote spaker), Indiana University, USA
"*Wolbachia* utilize actin for maintenance and transmission within *Drosophila melanogaster*"
15:25-15:50 Ewa Chrostek (keynote spaker), Instituto Gulbenkian de Ciência, Portugal
"Mutualism breakdown by amplification of *Wolbachia* genes"
15:50-16:10 Rupinder Kaur, Medical University of Vienna, Austria
"Symbiosis and Immunity: spatio-temporal infection dynamics of *Wolbachia* and RNA viruses in *Drosophila suzukii* and related species"
16:10-16:30 Florence Gutzwiller, University of Manchester, UK
"Dynamics of *Wolbachia pipientis* gene expression across the *Drosophila melanogaster* life cycle"

Poster presentations

P82. Amelia Lindsey, University of California, USA
"Comparative genomics of *Wolbachia*: evolution of genome content in a reproductive parasite"
P83. Barton Slatko, New England Biolabs, USA
"The fellowship of the ring: is heme biosynthesis an influencing factor in *Wolbachia*-filarial nematode endosymbiosis?"
P84. Kathy Sheehan, Indiana University, USA
"*Wolbachia* evolution in mutant *Drosophila* hosts"
P85. Sergios-Orestis Kolokotronis, Fordham University, USA
"*Wolbachia* wCle discovery in the bedbug genome"

Friday 17th - Room 3.2.14

Connecting habitats (CHA)

Christian Printzen (Senckenberg Research Institute and Natural History Museum, Germany)

Oral presentations

17:15-17:40 Fernando Fernandez-Mendoza, Karl-Franzens-Universität Graz, Austria
“Fundamental vs. realized association niches. Understanding the patterns of photobiont association in *Cetraria aculeata*”
17:40-18:00 Ranjeet Bhagooli, University of Mauritius, Mauritius
“*Symbiodinium* clade distribution in *Acropora muricata*: implications for physiological performance”
18:00-18:20 Nicole Lopanik, Georgia State University, USA
“Contrasting host and symbiont biogeography in a marine defensive symbiosis”
18:20-18:40 Sushma Mattan-Moorgawa, University of Mauritius, Mauritius
“Variable stress responses of tissue balls from hard corals harbouring clades C and D symbionts”
18:40-19:00 Tetiana Lutsak, Senckenberg Research Institute and Natural History Museum Frankfurt, Germany
“Mycobiont-photobiont interactions of the lichen *Cetraria aculeata* in high alpine regions of East Africa and South America”

Poster presentations

P86. Lucia Muggia, University of Trieste, Italy
“Insights in the lichen-formig species complex *Tephromela atra*: mycobiont-photobiont specific association defines a new taxon”
P87. Ranjeet Bhagooli, University of Mauritius, Mauritius
“Differential thermal stress responses of scleractinian corals habouring similar ITS2 *Symbiodinium* symbionts”
P88. Sushma Mattan-Moorgawa, University of Mauritius, Mauritius
“Temperature and light effects on the symbiont photo-physiology, density and clade diversity in the coral *Acropora muricata* from different zones of a tropical reef”
P89. Yohan Louis, University of Mauritius, Mauritius
“Seasonal zooxanthellar clade C photo-physiology in *Acropora muricata* colonies with different light and thermal histories”

Friday 17th - Room 2.2.15

Reticulate evolution before and after the modern synthesis: historical and epistemological perspectives and wider applications beyond traditional fields (REB)

Nathalie Gontier (Universidade de Lisboa, Portugal)

Jan Sapp (York University, Canada)

Oral presentations

14:30-15:00 Ricardo Guerrero (keynote speaker), BKH-Academia Europaea, Spain
“From the cell to the ecosystem: the physiological evolution of symbiosis”
15:00-15:30 Eric Bapteste (keynote speaker), CNRS, IBPS, UPMC, France
“Network-thinking: a complementary perspective to address the compelling epistemological and biological challenges raised by reticulate evolution”
15:30-16:00 Francisco Carrapiço, Universidade de Lisboa, Portugal
“The symbiogenic superorganism concept. An old new problem for the neo-Darwinian synthesis?”
16:00-16:30 Laura S. Weyrich (keynote speaker), University of Adelaide, Australia
“Untangling the evolutionary history of the human microbiome using Neanderthal dental calculus: cultural and environmental impacts on human health, disease, and evolution”

16:30-17:15 Coffee break

17:15-17:45 Vitor G. Faria & Élio Sucena (keynote speaker), Instituto Gulbenkian de Ciência, Portugal
“Intracellular endosymbiont selection contributes to *Drosophila* adaptation to viral infection”
17:45-18:15 Luís Correia (keynote speaker), University of Lisbon, Portugal & António Manso (keynote speaker), Tomar Polytechnic Institute, Portugal
“Modeling Symbiosis: A multiset model of multi-species evolution”
18:15-18:45 Davide Vecchi, University of Lisbon, Portugal
“A symbiotic view of biological individuality”
18:45-19:00 Nathalie Gontier (keynote speaker), Universidade de Lisboa, Portugal
“Book Launch: Reticulate evolution: Symbiogenesis, lateral gene transfer, hybridization, and infectious heredity”

Friday 17th 16:30-19:00

Teaching symbiosis: look at successful strategies

Marc-André Selosse (Muséum National d’Histoire Naturelle, France)

Silvana Munzi (Universidade de Lisboa, Portugal)

Gregory Crocetti & Briony Barr (Scale Free Network, Art-Science Collaborative, Australia)

Poster presentation

Prof. Ana Fraga, Maria Luísa Amaral, Matilde Quintana, Margarida Fonseca, Joana Jerónimo, Catarina Carvalheiro, M. Ana Menezes, 5° ano (11 years old students) Colégio Mira Rio, Lisbon, Portugal
“A influência dos fatores abióticos no comportamento das minhocas (The influence of abiotic factors on earthworms behavior)

Model displays

Francisco Carrapiço, Universidade de Lisboa, Portugal
“*Azolla* nitrogen fixing symbiosis”
François Lallier (UPMC-Univ Paris 6, France)
“Deep-sea symbioses – movie”
Filip Husnik (University of Montana, USA)
“Tripartite nested symbiosis in mealybugs”
Patricia P. Stock (University of Arizona, USA)
“Entomopathogenic nematodes and their symbiotic bacteria”
Marc-André Selosse (Muséum National d’Histoire Naturelle, France)
“Tree domatia and symbiotic mices”
Sónia Duarte (LNEC, Portugal)
“Flagellate protists of termites”
Teresa Lino-Neto (University of Minho, Portugal)
“Ectomycorrhizae”
Francisca Rodrigues dos Reis (University of Minho, Portugal)
“Orchid mycorrhizae”
Paola Furla (Université de Nice-Sophia Antipolis)
“Xanthellae-Cnidirians”
Silvana Munzi (Universidade de Lisboa, Portugal)
“Lichens”
Lisl Esherick (Stanford University School of Medicine, USA)
“The symbiotic sea anemone *Aiptasia*”
Fabrice Not and Xavier Bailly (Station Biologique de Roscoff, France)
“*Symsagittifera roscoffensis* and its algal symbionts”
“Phototrophic symbioses in plancton”
Sven Gould (University of Dusseldorf, Germany) and Cessa Rauch (Heinrich-Heine-University, Germany)
“*Elysia timida* and its kleptoplastids”
Manuela Giovannetti, Alessandra Pepe (University of Pisa, Italy) and Cristiana Sbrana (CNR, Italy)
“Arbuscular mycorrhizal networks”
Florent Masson (INSA de Lyon, France)
“*Sitophilus oryzae*, the cereal weevil, and its symbionts”
Briony Barr and Gregory Crocetti (Scale Free Network, Art-Science Collaborative, Australia)
“Storybooks featuring Squid-*Vibrio fischeri* and Hard Coral-Zooxanthellae-Rhizobia symbioses”
Louis Tisa (University of New Hampshire, USA)
“Actinorhizas: *Frankia* and plants”
Katherine Muller (University of Minnesota, USA) and Catherine Masson (INRA, France)
“Legumes and their nodules”

Friday 17th - Room 3.2.14

19:10-19:30 Closing session

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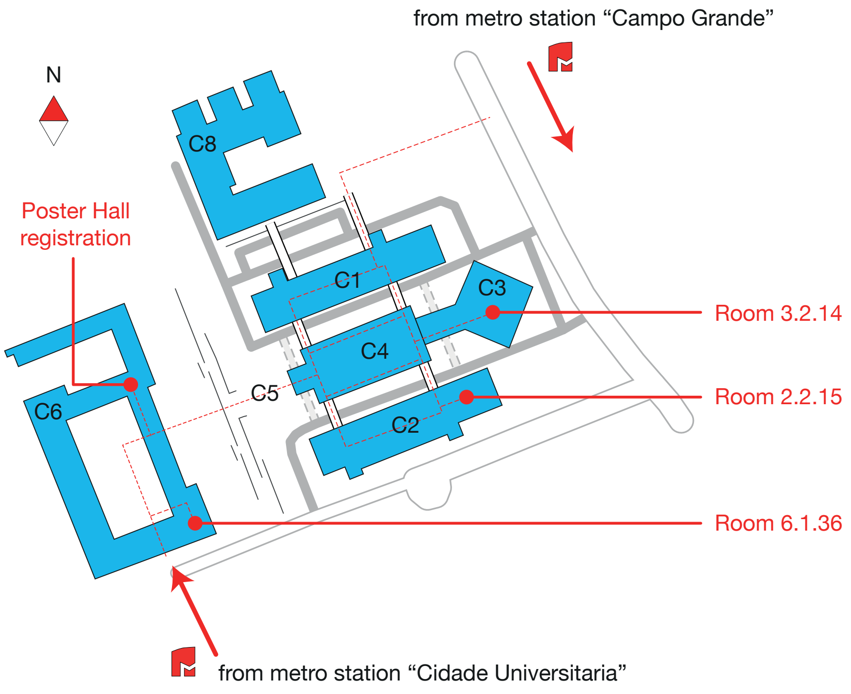

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Day: Monday 13th
Time: 12:00 - 13:00
Room: 3.2.14

Wine, witches and wilderness: The sculpting of civilization by microbial symbionts

RUSTY RODRIGUEZ

Adaptive Symbiotic Technologies, Seattle, Washington USA

The development of microbial photosynthesis 3.4BYA is theorized to be responsible for the accumulation of atmospheric oxygenic sufficient for aerobic respiration which began approx. 1-2 BYA. That led to an explosion of biological diversity eventually resulting in the emergence of land plants and animals. Fossil records indicate that when plants moved onto land approx. 450 MYA, they were intimately associated with filamentous fungi. Although Symbiosis was not defined until 1865 by Anton DeBary, there are many examples throughout history of how symbiosis sculpted civilization. The symbiotic continuum spanning from mutualism to parasitism has collectively been responsible for some of the most beautiful and horrific events in history, each of which altered the future. In recent times, it has become clear that symbiosis has also sculpted plant and animal life on earth. Since DeBary's remarkable description, it has become clear that all plants and animals are symbiotic with communities of microorganisms and that the vast majority of these interactions are non-pathogenic. In fact, it appears that plants and animals cannot survive without their microbial communities which play important roles in their ecology, adaptation, and fitness. I will discuss how symbiosis has sculpted civilization through agricultural development, human behavior and ecosystem health, and the potential of symbiosis for future sustainability.

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Day: Tuesday 14th
Time: 12:00 - 13:00
Room: 3.2.14

The evolutionary persistence of sex and aging - Why NeoDarwinists are wrong about group selection on a symbiotic planet

DORION SAGAN

Four billion years of evolution on a crowded planet have made life on Earth richer than accounted for in neo-Darwinism. In this talk I take a look at the new biology, focusing on aging and the need to re-introduce group selection to explain aging as an internal process. Looking at older views, including the very popular but factually flawed Free Radical Theory, as well as the more sophisticated and evolutionary theories begun by Sir Peter Medawar, I argue for a rapprochement between mainstream Darwinism and newer biological approaches with their focus on symbiosis, microbiome, and interspecies relationships. A new theory, better in accord with the facts—The Black Queen hypothesis, also the subject of a new coauthored book—better explains senescence. Like the Red Queen theories that have been accepted by neo-Darwinism to explain the persistence of meiotic reproduction, the Black Queen theory invokes selection at the population level. In this talk I will discuss the work of Lynn Margulis, my mother and writing partner for thirty years, as well as the neo-Darwinist William D. Hamilton and the lepidopterist (and novelist) Vladimir Nabokov. Sexual reproduction seems to have evolved from protist cannibalism, and perhaps been maintained in part because of the protection it afforded against epidemics. Likewise, senescence, evolving from apoptosis and replicative senescence in protists, seems to have been selected for its ability to mitigate dangerously fast population growth.

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Replaying the evolution of rhizobia: adaptation to the symbiotic lifestyle

CATHERINE MASSON-BOIVIN

Laboratory of Plant Microbe Interactions (LIPM), UMR CNRS-INRA 2594/441, BP 52627, 31326 CASTANET TOLOSAN CEDEX , France

The nitrogen-fixing legume-rhizobium symbiosis is an evolutionary novelty of extreme biological and ecological importance, as a major contributor to the global nitrogen cycle. Nowadays it is serving as a model for transferring nitrogen fixing capacities to non legume crops. Rhizobia are remarkable examples of phylogenetically dispersed bacteria that, although achieving a complex biological function, arose via horizontal gene transfer of only a few key symbiotic genes in different genetic backgrounds. Rhizobia are currently distributed in hundreds species belonging to 13 saprophyte- and pathogen-containing genera of alpha- and beta-proteobacteria. Rhizobial mutualistic symbiosis with legumes is a complex process involving three main steps, nodule organogenesis, intracellular infection and nitrogen fixation. This endosymbiosis is controlled by a large number of genes in both partners including a set of essential nodulation and nitrogen fixation bacterial genes clustered in mobile genetic elements such as symbiotic plasmids or genomic islands. Successful transfer over large phylogenetic distances has been rare, likely because recruitment of bacterial functions and adaptation to the plant host need extensive genome reprogramming .To get insights into the molecular and evolutionary mechanisms that facilitated the long distance spread of symbiotic genes, we experimentally replayed the evolution of rhizobia. Following introduction of the symbiotic plasmid of Cupriavidus taiwanensis, the Mimosa symbiont, into pathogenic Ralstonia solanacearum we challenged transconjugants to become Mimosa symbionts through serial and parallel ex planta-in planta (Mimosa) passages. This alternation recapitulated the shifts between free-living and symbiotic lives that have shaped the natural evolution of rhizobia. Evolution was surprisingly fast since the first two major symbiotic steps, nodulation and intracellular infection, were not only activated but also dramatically improved over 17 cycles (~400 generations) in all lineages. Evolution relied on many genomic changes and involved a mechanism of transient hypermutability that accelerates the symbiotic adaptation process under selection pressure from the host plant.

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Day: Friday 17th
Time: 12:00 - 13:00
Room: 3.2.14

The symbiotic self in a Darwinian world

JAN SAPP

York University, Canada

The classical one genome-one organism conception is giving way today to a symbiotic conception of the organism. Research on the importance of symbiosis as a source of evolutionary innovation has been carried out for over 100 years close to the margins of biology and in virtual conflict with biology’s central doctrines. In this presentation I shall explore why hereditary symbiosis and the microbial world were not included in the neo-Darwinian evolutionary synthesis of the last century. I will explore why recognition for the importance of symbiosis in evolution has emerged in recent years, how molecular phylogenetic methods applied to the microbial world have led to a conception of a universal web of life, based on horizontal gene transfer and symbiosis, which transcends Darwinian conceptions and contradicts its central tenets, and how the study of microbiomes have further reinforced the organismal conception of the symbiome.

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Session Chairs: François Lallier[1], Jillian Petersen[2]
Chair affiliations: [1]UPMC-Univ Paris 6, France
[2]Max Planck Institute, Germany

Chemosynthetic symbioses in the genomic and post-genomic era

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Session: Chemosynthetic symbioses in the genomic and post-genomic era
Presentation type: Oral
Name, Contact: Cambon-Bonavita Marie-Anne, macambon@ifremer.fr

O

Rimicaris exoculata toward better understandings of a complex symbiosis

MARIE-ANNE CAMBON-BONAVITA[1] , LUCILE DURAND[1], MATHIEU GURI[2,4], JULIE PONSARD[3], CYRIELLE JAN[4], VALÉRIE CUEFF-GAUCHARD[1], JULIE REVEILLAUD[1], MOHAMED JEBBAR[4], PHILIPPE COMPÈRE[3], BRUCE SHILLITO[5] AND MAGALI ZBINDEN[5].

[1] *Ifremer, Laboratory of Microbiology of Extreme Environments, LM2E, UMR 6197, ZI de la pointe du Diable, CS10070, 29280 Plouzané, France*
[2] *CNRS, LMEE, Technopôle Pointe du diable, BP 70, 29280 Plouzané, France*
[3] *Université de Liège, Département de Biologie*

Rimicaris exoculata is a hydrothermal shrimp that dominates the megafauna of some Mid Atlantic ridge sites. This species harbors a complex community of bacterial epibionts inside its gill chamber and digestive tract. To describe these microbial communities, several approaches have been used, microscopy (SEM, TEM, FISH), molecular biology (16S diversity, functional genes research, metagenomic), as well as in vivo experiments. Our data indicated wider gill chamber diversity than previously assumed with Epsilonproteobacteria lineages but also occurrence of Gammaproteobacteria (two lineages) and Zetaproteobacteria. We observed bacterial intracellular sulfur- and iron-enriched granules and some methanotrophic-like bacteria cells. A shift is observed between the egg microbial population and the adult one, indicating that part of the epibiotic community is already on eggs. Genes characteristic of methane-oxidizing (pmoA) and sulfide-oxidizing (APS) bacteria, were also identified. A metagenomic approach revealed the carbon dioxide fixation via the rTCA and CBB cycle and results suggest that three metabolic types (iron, sulfide and methane oxidation) would co-occur within this community. Moreover genes implied the host-symbiont recognition pathway have been revealed. A current metagenomic approach using the Illumina platform is aiming at reconstructing the symbiotic genomes. According to in vivo experiments, evidence of trophic exchanges from bacteria to the host was established by the first direct demonstration of in vivo nutritional transfers, this association being thus regarded as a true mutualism. The import of soluble microbial by-products does occur by permeation across the gill chamber integument rather than by the digestive pathway. Regarding the gut microbial community, our results indicated a wider permanent diversity than previously assumed and the presence of long microbial filaments directly in contact with gut epithelial cells inserted between microvillosities.

Acknowledgements: The authors thank Ifremer, Région Bretagne, Ouest Genopole, ANR deepoases/Carnot, CNRS, UBO, Belgian NFS Research, LabexMER and MAMBA project for their financial support.

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Using proteomics to better understand a symbiosis in deep sea hydrothermal vent

DÉTRÉE CAMILLE[1], TANGUY ARNAUD[1], VINH JOELLE[2], HADDAD IMANN[2], DEMEY EMMANUELLE[2], LALLIER FRANÇOIS[1], MARY JEAN[1]

[1] *Adaptation et Biologie des Invertébrés en Conditions Extrêmes, UMR 7144, CNRS-UPMC, Station Biologique de Roscoff, Place Georges Teissier 29680 Roscoff, France,*
[2] *USR 3149 Ecole Supérieur de Physique et Chimie Industrielle de Paris 10, rue Vauquelin*

Hydrothermal vents are located on the mid-ocean ridges, and are characterized by challenging physico-chemical conditions (high temperature, acidic pH, high concentrations of reduced compounds and metals) (Cavanaugh *et al.*, 2006). Despite these conditions dense hydrothermal communities develop down around hydrothermal fluid emissions. The presence of marine invertebrates relies on their capacity to cope with these challenging factors, and, for those forming most of the biomass, on their ability to live in symbiosis with chemoautotrophic bacteria. *Bathymodiolus azoricus* is one of these symbiotic species that harbors two types of γ-proteobacteria, a sulfide-oxidizing bacterium (that uses the oxidation of H_2S as the source of energy and CO_2 as source of carbon) and a methane-oxidizing bacterium (that uses the oxidation of CH_4 as both a source of energy and carbon) (Fiala-Medioni *et al.*, 2002). These bacteria are located in specific epithelial cells in the gill tissue of the mussel. Furthermore, the proportion and number of these symbiont types in *B. azoricus* can change in response to environmental conditions, and especially on the relative concentration of reduced compounds. The aim of our study is to understand molecular mechanisms of acquisition, regulation and maintenance of the symbiotic charge in *B. azoricus* gills through a proteomic approach. Experiments on the field were carried out, to deplete mussels from their symbionts and compared to natural population. Proteomic analyses were carried out by using SDS PAGE for protein separation followed by nano-LC-MS/MS for the identification and quantification of proteins. These analyses allow us to find immunity proteins that can also be involved in some symbiosis mechanism.

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Revisiting the flexible symbioses of deep-sea mussels using NGS, FISH and live experiments

SÉBASTIEN DUPERRON[1,2], SVEN R. LAMING[1], KAMIL M. SZAFRANSKI[1], BÉRÉNICE PIQUET[1], ADRIEN QUILES[1], BRUCE SHILLITO[1], FRANÇOIS H. LALLIER[3]

[1] *Sorbonne Universités, Université Pierre et Marie Curie, UMR 7208, Adaptation aux Milieux Extrêmes –Paris, France*
[2] *Institut Universitaire de France, Paris, France*
[3] *Sorbonne Universités, Université Pierre et Marie Curie, UMR 7144, ABICE*

Bathymodioline mussels are efficient colonizers of deep-sea reducing habitats including hydrothermal vents, cold seeps, wood and whale falls. They owe their success to their efficient symbiotic associations with one-to-several lineages of bacteria that fulfill most of the mussel's nutritional requirements. In recent years, mussel symbioses involving multiple bacterial partners have been shown to be highly flexible at several scales. We present a summary of recent research investigating this flexibility. Studying the diversity of symbionts in different mussel species, we discovered that symbioses could disappear over short evolutionary timescales. By comparing the composition of symbiont communities among different populations of a single Idas mussel species spanning the north eastern Atlantic and Mediterranean, we realized that a host species could harbor different symbiont assemblages depending on its geographical origin. To test this flexibility at the scale of a single individual, we performed experiments on live hydrothermal vent Bathymodiolus maintained in pressurized aquaria and exposed to various environmental conditions. These demonstrated that symbiont populations quickly adapt to environmental variations, and that a dual symbiosis involving sulphur- and methane-oxidizers could almost turn into sulfur-oxidizing-only symbiosis in a very short time under moderately high sulfide. Compared with other metazoan groups displaying chemosynthetic symbioses, Bathymodioline mussels are able to associate with a broader phylogenetic and metabolic diversity of bacteria. Besides, these symbioses are highly flexible at various scales. Altogether, these features may be key to the success of deep-sea mussels in the various habitats they colonize.

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Metabolic variability between symbiont subpopulations in *Riftia pachyptila*

TJORVEN HINZKE

Institute of Marine Biotechnology, Germany

The giant tube worm *Riftia pachyptila*, an invertebrate living at hydrothermal vents, depends entirely on one species of sulfur-oxidizing chemosynthetic bacteria for nutrition. These bacteria live in the worm's trophosome, an organ which is, instead of an intestinal tract, located in the coelomic cavity. Particularly puzzling about this intimate symbiosis is the broad spectrum of metabolic pathways employed by the symbionts: Some reactions are seemingly redundant – such as the two CO_2 fixation mechanisms Calvin cycle and rTCA cycle – or even opposed to each other, but are expressed in the same symbiont population in one host, as revealed by previous proteomic analyses. This might be related to the symbionts' cell cycle-dependent differentiation from dividing rods into small and subsequently larger cocci in the trophosome lobules. These "morphotypes", representing distinct subpopulations, probably exhibit individual metabolic profiles. Therefore, morphotype-dependent metabolic differences are likely to exist between symbiont subpopulations, resulting in the observed metabolic versatility. Culture-independent techniques are the method of choice for investigating this hypothesis, as the symbiont is not cultured to date. For proteomic studies, fresh trophosome homogenate was subjected to density gradient centrifugation to separate the morphotypes. The generated fractions are analyzed via CARD-FISH to count and measure the symbiont cell shapes and sizes. 1D gel-based and gel-free proteomic approaches are used for comprehensive analyses of enriched fractions. Furthermore, tissue was fixed for immunohistochemical analyses to visualize the distribution of metabolic key enzymes in trophosome lobule sections with labelled antibodies.As confirmed by CARD-FISH, different symbiont morphotypes were successfully enriched, and are now individually accessible by proteomics. These analyses are expected to reveal differences between the relative usage of metabolic pathways in the subpopulations of the *Riftia*-symbiont and to shed light on the reasons for the vast metabolic versatility of this bacterial species.

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The peptidoglycan recognition protein, a potential candidate for the regulation of the chemosynthetic symbiosis in the deep-sea hydrothermal vent mussel *Bathymodiolus azoricus*

CAMILLE DÉTRÉE[1], ARNAUD TANGUY[1], FRANÇOIS LALLIER[1], JEAN MARY[1]

[1] *Adaptation et Biologie des Invertébrés en Conditions Extrêmes, UMR 7144, CNRS-UPMC, Station Biologique de Roscoff, Place Georges Teissier 29680 Roscoff, France*

Bathymodiolus azoricus is a hydrothermal vent mussel that harbors in its gill tissue two types of symbiotic bacteria: sulfide-oxidizing (that uses the oxidation of H_2S as the source of energy and CO_2 as source of carbon) and methane-oxidizing (that uses CH_4 as both a source of energy and carbon) (Fiala-Medioni *et al.*, 2002). Using a proteomic approach, we identified proteins potentially involved in the regulation of symbiosis (cf. Détrée *et al.* abstract “Using proteomics to better understand a symbiosis in deep sea hydrothermal vent”). Among these proteins the peptidoglycan recognition proteins (PGRP) are interesting candidates. Peptidoglycan recognition proteins have been described as innate immunity molecules in numerous invertebrates, especially in mollusks. These proteins recognize the peptidoglycan of gram (-) bacteria and induce subsequent activation of signaling pathway for immune response. Moreover, Bettencourt *et al.* (2014) showed, using quantitative PCR, that the PGRP encoding gene presented a high expression level in gill tissues of *B. azoricus* collected at the Menez Gwen site. Our analysis of the gill transcriptome of *B. azoricus* revealed at least four different PGRP transcripts. The aim of this study is to determine the phylogenetic relationship between these isoforms and, by sequence analysis and 3D structure modelling, to infer subcellular location and function for the different isoforms of PGRP.

Bettencourt R., Rodrigues M., Barros I., Cerqueira T., Freitas C., Costa V., Pinheiro M., Egas C. and Santos, R.S. (2014): *Fish & Shellfish Immunology 39* : 343-353.
Fiala-Medioni, A., McKiness, P., Dando, P., Boulegue, J., Mariotti, A., Alayse-Danet, A.M.,Robinson. J.J., Cavanaugh, C.M. (2002): *Marine Biology 141*: 1035–1043.

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The power of 'omics' for revealing hidden metabolic potential and cryptic host-symbiont interactions in chemosynthetic symbioses

JILLIAN PETERSEN

Max Planck Institute for Marine Microbiology, Celsiusstrasse 1, 28359 Bremen, Germany

Chemosynthetic symbioses between bacteria and marine invertebrates have been known to science for nearly forty years. We now know that on the host side, these associations have evolved multiple times in convergent evolution in at least nine animal groups. Similarly, chemosynthetic symbionts have evolved – and are continuing to evolve - from numerous bacterial lineages. Genomics is rapidly changing our understanding of the energy sources used by chemosynthetic symbionts to power their metabolism. For the first thirty years after their discovery, only two energy sources were known to power chemosynthetic symbioses: (1) reduced sulfur compounds, used by sulfur-oxidizing symbionts, and (2) methane, used by methane-oxidizing symbionts. Only a few studies have examined the genomic potential for other energy sources and even fewer have investigated which energy sources are used *in situ*. We recently discovered that chemosynthetic symbionts use a surprisingly wide range of energy and carbon sources that includes hydrogen and organic compounds. In my talk, I will give an overview of our recent discoveries from metagenomics, metatranscriptomics and metaproteomics studies, focusing on the bivalve *Bathymodiolus* symbioses. With at least 30 so-far described host species and a world-wide distribution at hydrothermal vents and cold seeps, these symbioses are emerging as ideal models for comparative ecological and evolutionary studies. I will show how 'omics' have revealed novel symbionts, novel symbiont energy sources, and the molecular basis of host-symbiont interactions in Bathymodiolus. I will also discuss the role of horizontal gene transfer in symbiont genome evolution and the acquisition of novel metabolic capabilities.

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Apoptosis and symbiosis in bivalve molluscs

ANN C. ANDERSEN[1], MARIE LOUVIGNÉ[1], HAYAT GUÉZI[1], KAMIL SZAFRANSKI[2], BRUCE SHILLITO[2], SÉBASTIEN DUPERRON[2], FRANÇOIS H. LALLIER[1], ARNAUD TANGUY[1]

[1] *Sorbonne Universités, Université Pierre et Marie Curie, CNRS, UMR 7144 Station Biologique, F-29680 Roscoff, France.*

Apoptosis, or programmed cell death, occurs during development and tissue renewal in response to internal or external signals. It has been evidenced in the gills of symbiotic bivalves such as the mangrove lucinid *Ctena* (Codakia) *orbiculata* (Elisabeth, 2012) or the deep-sea mytilids *Bathymodiolus thermophilus* – from hot vents – and *B. boomerang* – from cold seeps (Guezi, 2013). The bacteriocytes composing a large part of the gill epithelium of these bivalves shelter chemotrophic symbiotic bacteria that can oxidize either sulfides or methane to fix carbon and feed their host. Apoptosis could be a process used by the host to regulate the population of symbiotic bacteria in its gills. A large-scale transcriptomic survey has shown (Guezi, 2013) that in *B. thermophilus* gills with a heavy load of symbiotic bacteria, the expression of genes related to apoptosis inhibition is lowered and, conversely, genes promoting apoptosis are over-expressed. We have confirmed this finding by using microscopic observations with TUNEL assay of the gills from individuals with contrasted bacterial loads. In B. thermophilus, the number of apoptotic cells range from 10% in individuals with a small bacterial load, up to 30% for those with the heaviest load. But in B. boomerang this proportion rises to 80%, questioning the relevance of these results.

Elisabeth, N.H., Gustave, S.D.D. and Gros, O. (2012). Is apoptosis really linked to symbiosis, or is it an artifact caused by the sampling stress of depressurization in these deep-sea mussels? Microscopy Res. Tech. 75 (8): 1136–1146.Guézi, H.
Adaptation au mode de vie symbiotique chez les moules du genre Bathymodiolus des écosystèmes chimiosynthétiques profonds : approche transcriptomique. (2013). Thèse en Biologie de l'Université Pierre et Marie Curie - Paris 06. ED Interdisciplinaire pour le vivant.

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Session: Chemosynthetic symbioses in the genomic and post-genomic era
Presentation type: Poster
Name, Contact: Le Bloa Simon, simon.le.bloa@ifremer.fr

P

Toward a better understanding of the symbiotic relationships in *Rimicaris exoculata* model

SIMON LE BLOA[1], LUCILE DURAND[2], LAURE TAUPIN[3], CHARLOTTE MARTEAU[3], BRUCE SHILLITO[4], MAGALI ZBINDEN[4], MARIE-ANNE CAMBON-BONAVITA[1], ALEXIS BAZIRE[3]

[1] *UMR 6197 - Laboratoire de Microbiologie des Environnements Extrêmes (LM2E)* Ifremer Centre Bretagne - CS 10070 Plouzané, 29280 France

The caridean shrimp *Rimicaris exoculata* dominates the fauna at several Mid-Atlantic Ridge hydrothermal vent sites. It has two distinct microbial communities whose roles remain partly understood. One of these ectosymbiotic bacterial communities is associated with mineral oxide deposits and colonizes its enlarged gill chamber. Its structure and establishment process reminds of biofilm ones which use a communication pathway dependent on the bacterial density called Quorum Sensing (QS). Until now, their acquisition is not fully understood. The presence and expression of genes involved in the QS was studied by PCR, RT-PCR and QPCR. The presence of the luxS gene in the epibiontic community of R. exoculata at different moult stages was confirmed for the Rainbow and TAG vent sites. The RT-PCR experiments were unable to highlight a potential activity of QS only for shrimps of the TAG and Rainbow vent sites. Phylogenetic analysis has shown the presence of 3 haplotypes for luxS genes (Epsilonproteobacteria) and luxR (Gammaproteobacteria) distributed according to the Rainbow, TAG and Snake Pit vent sites. This suggests that these genes could be used as biogeographical genetic marker of these symbiontic populations. Preliminary Q-PCR experiments suggested a correlation between the expression of luxS gene and the development of the epibiont affiliated to the Epsilonproteobacteria. The characterization and quantification of communication molecules (acylhomoserine lactones (AHLs)) have been done by LC-MS-MS. No AHLs could be highlighted in this study.

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Marine chemoautotrophic symbioses examined by physiological proteomics

STEPHANIE MARKERT

Institute of Marine Biotechnology Greifswald, Germany

Many deep-sea hydrothermal vent animals, such as the giant tube worm *Riftia pachyptila*, and mussels of the genus *Bathymodiolus*, live in highly intimate symbioses with chemoautotrophic bacteria. While the general mechanism that fuels these mutually beneficial associations is widely known, other important key questions have remained unanswered, e. g.: Which factors promote the symbionts' colonization of host tissue, but prevent infection by other bacteria? How are facultative symbionts specifically adapted to two very dissimilar life styles, inside the host and in the environment? Are there subpopulations within the symbiont community in one host, which might be relevant for the maintenance of the symbiotic system? Conventional physiological investigations are impeded by the fact that the bacterial partners have escaped cultivation outside their hosts as yet. Using a culture-independent proteomic approach, however, circumvents this problem and allows for comprehensive and very detailed metabolic profiling. Using ESI-LC mass spectrometry we analyzed symbiont-containing tissues and enriched symbiont fractions from *R. pachyptila* and *B. azoricus* to yield and compare specific protein expression patterns. For the *B. azoricus* symbiosis an elaborate metabolic interdependency between the individual partners was revealed by this approach. A number of candidate symbiosis-specific proteins were identified, which mediate potential host-symbiont interactions, such as recognition, invasion and persistence. The results of our *Riftia* studies furthermore suggest that expression of metabolic pathways is highly adaptable to the prevailing energy situation. The *R. pachyptila* symbionts utilize both sulfide and thiosulfate as electron donors, they use both the reductive TCA cycle and the Calvin cycle for CO_2 fixation, and they are capable of using nitrate as an alternative electron acceptor besides oxygen. This enormous metabolic flexibility on the symbionts' side, combined with the host's ability to effectively buffer unsteady ambient conditions, might be the key to success for these remarkable symbioses.

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Genomic and population genetic analysis of gastropod symbionts in deep-sea hydrothermal fields

SATOSHI NAKAGAWA

Laboratory of Marine Environmental Microbiology, Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University Oiwake-cho, Kitashirakawa, Kyoto 606-8502, Japan

Deep-sea hydrothermal fields harbor the light-independent, highly productive ecosystems driven primarily by chemoautotrophs. Most of the invertebrates thrive there through their relationship with endo- or epi-symbiotic chemoautotrophs. Chemoautotrophs are microorganisms that are able to fix inorganic carbon using a chemical energy obtained through the oxidation of reduced compounds. Recent microbiological studies have led to an emerging view that the majority of deep-sea vent chemoautotrophs have the ability to derive energy from multiple redox couples in addition to the conventional sulfur-oxygen couple. Genomic, metagenomic and postgenomic studies have considerably accelerated the comprehensive understanding of molecular basis and evolution of deep-sea vent chemoautotrophy. For example, genomic analysis suggested that there were previously unrecognized evolutionary links between deep-sea vent chemoautotrophs and important human/animal pathogens. However, relatively little is known about the genome of gastropod endosymbionts. We have sequenced whole genomes of the probably horizontally transmitted endosymbionts of various large gastropod species, as an effort to address questions about 1) the genome evolution of horizontally transmitted, facultative endosymbionts, and 2) population genetic properties of gastropod symbionts. Genomes of gastropod symbionts generally display features consistent with ongoing genome reduction such as large proportions of pseudogenes and transposable elements. The genomes encode multiple functions for chemoautotrophic respirations, probably reflecting their adaptation to their niches with continuous changes in environmental conditions. When we compared different gastropod species, we found allelic variability of endosymbionts was significantly different with each other. We will also compare these genomic and population genetic characteristics of gastropod endosymbionts with those of free-living relatives.

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Tracking the life-cycles of deep-sea metazoan's bacterial symbionts at hydrothermal vents and cold seeps

KAMIL M. SZAFRAŃSKI[1,2,3], PHILIPPE DESCHAMPS[3], MARINA R. CUNHA[4], SYLVIE M. GAUDRON[1,5], SÉBASTIEN DUPERRON[1,2,6]

[1] *Sorbonne Universités, UPMC Université Paris 06, UMR 7208, Adaptation aux Milieux Extrêmes – Paris, France.*
[2] *UMR MNHN UPMC CNRS IRD UCBN 7208, Biologie des Organismes Aquatiques et Ecosystèmes, 75005 Paris, France.*
[3] *UMR8079 Unité d'Ecologie, Systématique et Evolution, CNRS UMR 8079, Université Paris-Sud, Orsay, France*

Chemosynthetic prokaryotes play a crucial role in energy and carbon cycling in the deep-sea. Using the energy of chemical bonds, they are able to convert inorganic carbon into organic molecules. That is why symbiotic associations with chemosynthetic bacteria allow some metazoans to thrive at hydrothermal vents and cold seeps. Bacterial symbiont transmission is a key step in the renewal of the symbiotic interaction at each host generation, and different modes of transmission can be distinguished. Understanding the mechanisms of symbiont transmission and the processes driving their diversity, colonization and dispersal are thus of prime importance for deep-sea biologists. In an attempt to document these symbionts life cycles, we investigated symbiont transmission in several bivalve species using fluorescence *in situ* hybridization microscopy. We also investigated the diversity and distribution of free-living bacteria closely related to chemosynthetic symbionts in standardized colonization devices filled with wood cubes or alfalfa grass and deployed over the last 10 years at vent and seep sites in the Norwegian Sea, the Mid-Atlantic Ridge, the Gulf of Cadiz and the eastern Mediterranean. Devices were recovered after different periods of deployment ranging from 2 weeks to 3 years, and bacterial diversity was analyzed by sequencing of 16S rRNA pyrotags. We confirmed intracellular localization of symbiotic bacteria within female gametes and the transovarial transmission of symbionts in Vesicomyidae clams. In parallel, the detailed characterization of bacterial assemblages colonizing plant-derived substrates yielded numerous bacteria closely related to known bacterial symbionts of metazoans, suggesting their abundance at some sites. Their occurrence patterns were similar to those of their respective hosts and only horizontally-transmitted chemosynthetic symbionts could be detected, suggesting their ability to cope with two very distinct lifestyles. These results shed new light on the diverse strategies employed by symbionts, which probably explain the success of these associations in various deep-sea habitats.

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Host-symbionts relationship in the deep-sea mussel *Bathymodiolus* spp explored with transcriptomic approaches: a state of the art

ARNAUD TANGUY[1], ISABELLE BOUTET[1], HAYAT GUEZI[1], ANN ANDERSEN[1], TAKESHI TAKEUCHI[1,2], EIICHI SHOGUCHI[2], NORI SATOH[2], LALLIER FRANÇOIS[1]

[1] *Adaptation and Diversity in the Marine Environment; UPMC-Univ. Paris 6 & CNRS; Station Biologique, Roscoff, France*
[2] *Marine Genomics Unit, Okinawa Institute of Science & Technology, Onna-son, Okinawa, Japan*

The most abundant animal species from deep-sea chemosynthetic ecosystems have developed a single or multiple symbiosis with either sulfide-oxidizing or methanotrophic bacteria. The establishment and performance of such "symbiotic lifestyle" implies delicately balanced relationships between eukaryotic cells and bacteria, at the boundary between the "simple" phagotrophic predation/digestion of bacteria by eukaryotes and the cell disease/disruption provoked by pathogenic bacteria. In deep-sea chemosynthetic mussels, populations of bacterial endosymbionts established in gill cells change in density and composition in response to varying chemical conditions in their environment. This flexibility remains difficult to explain on a molecular basis and few studies have been conducted on the effect of this variability on the physiological status of the mussel or the identification of the genes and metabolisms potentially related to symbiosis control in the mussel gill. Of course, several mechanisms are involved in the maintenance of these symbioses in order to keep them stable over time. The mussel must regularly renew its bacterial population through bacterial division or uptake from the environment, but must also ensure that these populations do not overgrow within host tissues/cells. The cellular coordination that maintains homeostasis or controls growth in symbiotic host tissues is extremely important for the management of the symbiotic populations, and factors involved in the inhibition or activation of apoptosis appears to play a central role. Using transcriptomic approaches, we have explored several of those mechanisms in three different *Bathymodiolus* species living around hydrothermal vents or cold seeps, and harboring single or dual symbiosis, analyzing results in relation to the symbiont content of each individual. We have evidenced that, depending on species, the physiological responses of mussels exhibit specific patterns highlighting a more complex picture than initially expected. The results of this study and some interesting outcomes of the characterization of *B. azoricus* genome will be presented.

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Phenotypic convergence and conservation of immune processes involved in the establishment of detoxifying symbiosis between coastal and hydrothermal annelids

LOLITA ROISIN[1], CLAIRE PAPOT[1], DIDIER JOLLIVET[2], CÉLINE WICHLACZ[1], VIRGINIE CUVILLIER[1], STÉPHANE HOURDEZ[2], SÉBASTIEN DUPERRON[3] AND AURÉLIE TASIEMSKI[1]

[1] *Université de Lille 1, CNRS UMR8198, Unité Evolution Ecologie et Paléontologie, Ecoimmunology of Marine Annelids group, Villeneuve d'Ascq, France*

Until recently, the epibiotic microflora of marine animals was often considered as a random consortium (McFall-Ngai 2008). However, it has increasingly been shown that this microflora corresponds to a specialized microbial community forming a specific and stable association with its host. In chemosynthetic environments such as hydrothermal sources and sulfides-rich sediments, certain marine organisms are regularly colonized by ectosymbiotic bacteria (Dubilier, Bergin *et al.* 2008). Here, we describe the association of the coastal annelid worm *Capitella* with the giant sulfur-oxidizer *Thiomargarita*, a bacterium also described as a free-living species or attached to the byssus of a hydrothermal mussel (Salman, Amann *et al.* 2011). The fact that *Thiomargarita* can be associated to *Capitella* worms or to hydrothermal mussels suggests that factors other than biogeography and host taxonomy intervene, and constitute selective pressures leading to a convergent interspecific association.In some measure, the association between *Capitella* and *Thiomargarita* phenotypically evokes the detoxifying epibiosis observed in the hydrothermal annelid *Alvinella pompejana*. In the latter, studies have shown that the filamentous microflora (in this case obligatory and constituted by a consortium of proteobacteria without any *Thiomargarita*) was controlled by an antimicrobial peptide (AMP) called alvinellacin (Tasiemski, Jung *et al.* 2014). Interestingly, the gene coding for alvinellacin is evolutionarily linked to the gene coding for capitellacin, the alvinellacin ortholog found in *Capitella* (Tasiemski, Jung *et al.* 2014). In this context, we here describe how and why the immune effector of *Capitella* may allow the association with a sulfide-oxidizing bacterium. Even if the fluctuating chemosynthetic environment appears as a key point in the establishment and the evolution of the symbiotic association, our data suggest that the conservation of immune effectors such as AMPs also appear as an interesting factor to understand the functional modalities that govern interspecific associations in both coastal and hydrothermal environments.

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Session Chairs: Francisco Dionisio, Luis Carvalho
Chair affiliations: Universidade de Lisboa, Portugal

Communication, symbiosis and behaviour

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Sophisticated communication controls root infection in the rhizobium-legume symbiosis

FERNANDO SORROCHE[1], ANNE-MARIE GARNERONE[1], AMANDINE GASTEBOIS[1], VÉRÉNA POINSOT[2], CATHERINE MASSON[1] AND JACQUES BATUT[1]

[1] *LIPM UMR INRA 441/CNRS 2594 Castanet-Tolosan cedex France*
[2] *IMRCP UMR5326 CNRS Univ Toulouse III. Toulouse France*

Legumes and phylogenetically diverse soil bacteria called rhizobia have co-evolved a facultative nitrogen-fixing symbiosis. In most legumes, establishment of the symbiosis begins with the entry of bacteria in the root epidermis via specialised structures called infection threads (ITs). In parallel, organogenesis of nodules takes place in the root cortex. Subsequently nodule tissues are intracellularly invaded by bacteria that differentiate into nitrogen-fixing bacteroids. Tight control of nodulation and infection is required to maintain the mutualistic character of the interaction. A first mechanism called AON (for Autoregulation Of Nodulation) controls nodule numbers in response to endogenous and exogenous cues.We have discovered recently another regulatory loop, coined AOI, that negatively controls infection, ie IT formation (Tian *et al.* PNAS USA 2012). Contrary to AON, AOI involves a sophisticated molecular communication between the two symbionts. As a result, bacteria inside nodules modulate root susceptibility to infection systemically, thus preventing further infection events by rhizobia still present in the rhizosphere. Contrary to pathogenic interactions, mutualistic symbioses require retrocontrol mechanisms, to stabilize the interaction. AOI illustrates the key role of communication between the two symbionts in achieving this goal.

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Establishment of symbiotic assemblage associated with polcilloporid coral: two years planting experiment

TEMIR A. BRITAYEV, YURY V. DEART, SOFIA S. ZVONAREVA, VICTOR N. MIKHEEV

A.N. Sertzov Institute of Ecology and Evolution RAS

All scleractinian corals are serving as hosts for different animals establishing with them symbiotic assemblages (SA). The most diverse symbiotic fauna is associated with branched corals like *Acropora* and *Pocillopora* species. Among symbionts obligate and facultative species are usually distinguishing. Many of obligate symbionts play vital role in maintaining of coral health. The main object of our research was to consider establishment of SA associated with *Pocillopora verrucosa* coral in two years planting experiment. We aimed also to respond the questions whether first colonizers were facultative or obligate symbionts, and whether assemblage formed on planted coral colonies was similar with that on natural ones. Coral fragments without macrosymbionts were planted on frames in the vicinity of Nhtrang city, Vietnam. After 6, 12, and 24 months of exposure colonies were detached, measured, and associated animals were identified and counted. During period of exposure from 6 to 24 months colonies volume increased from 1.1 to 6.9 dm^3, number of symbiotic species increased from 10 to 28 species, species richness, from 2.6 to 8.6 species per colony, and abundance, from 5.4 to 30.5 specimens per colony. Among first colonizers specialized obligate symbionts were dominated in terms of diversity and abundance, while to the end of the experiment these indexes for facultative symbionts exceeded that for obligate ones. Comparison of SA of planted and natural colonies demonstrated that all indexes were higher in natural colonies. Species composition of planted colonies differed from that of natural ones in absence or rarity of gastropod *Coralliophila* spp., hermit crabs *Calcinus* spp., ophiuroids, and fish *Paragoibiodon* spp. These observations suggest that process of SA establishment was not completed during 24 months exposure, or that environmental conditions for cultivated colonies differed from that of natural ones.

The study was supported by Russian Scientific Foundation, project No 14-14-01179.

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Cooperative interactions with bacteria aid in toxin resistance in the social amoeba *Dictyostelium discoideum*

DEBRA A. BROCK, W. ÉAMON CALLISON, DAVID C. QUELLER, AND JOAN E. STRASSMANN

Department of Biology, Campus Box 1137, Washington University in Saint Louis, One Brookings Drive, Saint Louis, Missouri, USA

Eukaryotes are dependent on beneficial microbes, yet can be killed by harmful ones. How have they evolved responses that protect themselves from harmful bacteria while coddling the beneficial ones? An ideal system for investigating fundamental principles of symbiotic relationships is a simple eukaryotic system that has close relationships with only a few bacteria at a time, a small microbiome. The social amoeba *Dictyostelium discoideum* offers such a system because some clones known as farmers carry bacteria through the social stage to use as future seed corn and as protection against competitors, while others do not. Most of their life, *D. discoideum* amoebae consume bacteria and divide mitotically. Under starvation, amoebae aggregate into a multicellular body which crawls to light, then forms a fruiting body of 20% dead stalk cells and 80% living spore cells. During the crawling stage, some cells called sentinel cells, pass from front to back of the slug picking up toxins and bacteria, functioning as a combined liver and innate immune system. We hypothesize that farmer sentinel cell numbers may be reduced because they also have to retain their helpful bacteria, a potentially costly tradeoff. In support, we found that farmers have considerably fewer sentinel cells compared to non-farmers but we did not find a fitness trade-off against harm from a toxin. Farmers produced the same number of spores with or without toxin exposure while non-farmers suffered significant harm. However, removal of farmer-associated bacteria from farmers and subsequent toxin exposure leads to fewer spores produced just as when non-farmers are exposed. Additionally, we found farmer and non-farmer spores were equally viable after toxin exposure. These data suggest that farmer-associated bacteria augment the role of sentinel cells. Thus, the advantages farmers gain from carrying bacteria include not just food and protection against competitors, but also protection against toxins.

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Transcriptional landscape of *Burkholderia terrae* BS001 populations upon confrontation with the soil fungus *Lyophyllum* sp. strain *Karsten*

IRSHAD UL HAQ*, JAN DIRK VAN ELSAS

Department of Microbial Ecology, Groningen Institute of Evolutionary Life Sciences (GELIFES), Nijenborgh 7, 9747 AG, University of Groningen, The Netherlands

A time course confrontation of *Burkholderia* terrae BS001 cell populations with the soil fungus Lyophyllum sp. strain Karsten on soil–mimicking agar plates revealed that strain BS001 modulates the expression of key genetic circuits as a response to both the soil–mimicking environment and the emerging fungal hyphae. The stationary–phase sigma factor RpoS, as well as other genes under its control, were highly expressed across the treatments over the whole temporal regime. Strikingly, strain BS001 apparently perceived the presence of fungal hyphae early on in the experiment, i.e. with the fungal hyphae still being at a distance of 15 mm. At the early experimental stages when there was no physical contact between the partners (T1 – d 3 and T2 – d 5), strain BS001 chemotaxis–related genes were strongly upregulated upon confrontation with the fungus as compared to the control. Furthermore, at T2 a gene encoding a SET domain containing protein (a potential type 3 secretion system secreted effector protein) was also upregulated. At the physical contact stage T3 (d 8), several genes, including the clustered genes AKAUv1_2870056 – AKAUv1_2870060, potentially involved in oxidative stress response, next to two genes predicted to encode short–chain dehydrogenases/oxidoreductases (SDR), were highly upregulated in the interactome. In contrast, genes potentially involved in stress responses, i.e. dnaE2 and pqqC, were downregulated. This study provides evidence for the contention that strain BS001, from a stress–dominated status resulting from the chemical conditions of bulk soil, shows an early and dynamic response to emerging fungal hyphae, which is characterized by dynamically–changing chemotaxis, metabolic signalling and oxidative stress–responsive behaviours.

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Self-recognition and incompatibility affect the structure and interconnectedness of mycorrhizal networks formed by different isolates within Glomeraceae

ALESSANDRA PEPE[1], CRISTIANA SBRANA[2], MANUELA GIOVANNETTI[1]

[1] *Dept. Agriculture, Food and Environment, University of Pisa, Italy*
[2] *Institute of Agricultural Biology and Biotechnology, CNR, Italy*

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts, which obtain sugars from their host plants, in exchange of mineral nutrients absorbed from the soil and translocated to the root system by extraradical hyphal networks spreading from colonized roots into the surrounding environment. The production of large and interconnected mycorrhizal networks increases fungal absorbing surface and foraging ability, facilitating nutrient flow to host roots and affecting plant performance. An in vivo bi-dimensional experimental system allowed the visualization and quantification of structural traits of intact mycorrhizal networks, showing a high level of interconnectedness, as a result of hyphal self-recognition and fusion (anastomosis) in AMF belonging to different families. Here we report a differential hyphal compatibility in three AMF species, *Rhizoglomus intraradices*, *Funneliformis coronatum* and *Funneliformis mosseae*, belonging to the family Glomeraceae, growing in symbiosis with the plant species *Lactuca sativa*, *Cichorium intybus* and *Valerianella locusta*. Fungal identity significantly affected network structural traits, such as hyphal density, explored area and total hyphal length, and anastomosis frequency, whereas host identity represented a significant factor only for hyphal density and length. Anastomosis frequencies within networks formed by *F. coronatum* were 1/10 to 1/8, compared with those detected in the other two species. Microscopic analyses of *F. coronatum* networks showed no hyphal interactions in 71.6-93.6% of contacts, and incompatible responses among contacting or fusing hyphae in 5.1-20.6% of contacts. Possible effects of the structure of *F. coronatum* mycorrhizal network on the establishment and functioning of AMF symbiosis is discussed.

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The molecular communication in cnidarian-dinoflagellate symbiosis

JOHANA REVEL[1], LIONEL MASSI[2], MOHAMED MEHIRI[2], LAURE CAPRON[2], ISABELLE FOURNIER[3], MICHEL SALZET[3], CECILE SABOURAULT[1]

[1] *Université de Nice-Sophia-Antipolis, UMR7138 Evolution Paris-Seine, Valrose, BP71, 06108 Nice Cedex 02, France;*
[2] *Université Nice Sophia Antipolis, UMR7272, ICN, 06108 Nice Cedex 2*
[3] *Université Lille 1, Laboratoire PRISM, INSERM U1192, 59655*

Coral reef ecosystems are renowned for their diversity and beauty. Their immense ecological success is due to an endosymbiotic association between a cnidarian host and photosynthetic dinoflagellates (*Symbiodinium*), hosted within the cnidarian gastrodermal cells. The main benefit of this interaction is the translocation of photosynthetically fixed carbon (photosynthates) by symbionts to their cnidarian host. Various environmental stressors such as elevated sea-water temperature may cause the symbiosis breakdown or “bleaching”, and ultimately the host death. To understand the molecular communication between the host and symbionts, we analyzed the metabolic diversity of the Mediterranean symbiotic sea anemone, *Anemonia viridis*. We used mass spectrometry analyses to characterize the metabolites and their distribution in the different cellular compartments (epidermis, gastrodermis and *Symbiodinium* cells). We determined molecules potentially exchanged between the partners, as well as their modification following a bleaching event. Lipids have been demonstrated to be largely affected by a thermal stress. Due to their major roles in biological membrane constitution and energy storage, a focus on lipid composition was performed to investigate the lipid trafficking between host and symbionts. For example, we especially attempted to identify lipid compounds that may bind the sterol-binding proteins Niemann-Pick C, which are key players of the symbiotic interaction. Recent imaging tools, such as Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI) were also used to precisely localize specific metabolites playing a role in symbiosis maintenance, and to follow their evolution during a bleaching event. In this study, we combined classical spectrometric analytical tools and MSI techniques in order to provide insights on both the origin and localization of key molecules involved in the symbiotic interaction.This work was supported by the French ANR grant ANR-12-JSV7-0009-01 (inSIDE project)

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The complementary production of antibiotics by the leech *Hirudo verbana* and its gut symbiont *Aeromonas veronii* reinforces their mutualistic association

AURÉLIE TASIEMSKI[1], FRANÇOIS MASSOL[1], VIRGINIE CUVILLIER-HOT[1], CÉLINE BOIDIN-WICHLACZ[1], EMMANUEL ROGER[2], FRANCK RODET[3], ISABELLE FOURNIER[3], FRÉDÉRIC THOMAS[4], MICHEL SALZET[3]

[1] *Unité d'Evolution, Ecologie et Paléontologie (EEP), CNRS UMR8198, Université de Lille 1, 59655 Villeneuve d'Ascq, France*

Understanding host-symbiont interactions requires the exploration of the interaction between symbiotic and pathogenic microorganisms, as well as assessing under which conditions host organisms consider the presence of such bacteria as an infection. One simplified way to unravel host-symbiont interactions is to use a model organism housing a simple microflora. Here, we investigate microbe-host and microbe-microbe interactions in the hematophagous medicinal leech, *Hirudo verbana*. The gut of the leech is characterized by a minimalist symbiotic association dominated by the bacterium *Aeromonas veronii*, a versatile bacterium which possesses alternative lifestyles beyond that of mutualism (free living waterborne, pathogenic for fishes and human). Our data show that both partners produce antibiotics asynchronously inside the digestive tract. During blood digestion, the abundant symbiont secretes substances that provide the host with an immune protection against pathogenic bacteria and also prevent colonization by competing microorganisms. In-between meals, the leech gut produces antibacterial substances for immunity purpose that seemingly protect the *Aeromonas* symbiont, not targeted by leech antibiotics, against competition from other bacteria present in the leech habitat. This study suggests that leeches use antibiotics to maintain gut homeostasis. It also highlights a reciprocal immune benefit based on an immune relay between the two partners. These data substantially reinforce the hypothesis according to which the association between *H. verbana* and *A. veronii* results from a selected mutualism.

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Frankia Genomics and Genome-guided approaches toward understanding the actinorhizal symbiosis

LOUIS S. TISA[1], SERGIO SVISTOONOFF[2,3], NICHOLAS BEAUCHEMIN[1], SHELDON HURST IV[1], TEAL FURNHOLM[1], REDIET OSHONE[1], VALERIE HOCHER[2], HASSEN GHERBI[2], CLAUDINE FRANCHE[2], DIDIER BOGUSZ[2]

[1] *Dept. of Molecular, Cellular and Biomedical Sciences, University of New Hampshire, USA*
[2] *Equipe SYAT , UMR DIADE, IRD, 911 avenue Agropolis, 34394, Montpellier Cedex 5, France*
[3] *LCM & LMI LAPSE, Dakar, Sénégal*

Frankia forms nitrogen-fixing symbioses with 8 different Angiosperm families, termed actinorhizal plants. Symbiotic interactions between *Frankia* and the host plant are not well understood and very little is known about the initial molecular interactions in the rhizosphere. The nature of the chemical signals exchanged between the two partners of actinorhizal symbioses is still unknown. Due to the absence of genetic tools for *Frankia*, we have also pursued new genomic approaches toward studying these bacteria. Eighteen *Frankia* genomes have sequenced providing opportunities to use bioinformatics approaches and other new technologies. A correlation between genome size and plant host range was suggested from these data. Larger genome had broader host ranges. The absence of obvious nodulation genes similar to those found in Rhizobia genomes suggests that the actinorhizal symbiosis uses novel signal compounds during the infection process. Analysis of the *Frankia* genomes also demonstrated the presence of unexpected numbers of secondary metabolite gene clusters and potential novel natural products as candidates. Comparative genomic analysis will be discussed in terms of metal resistance mechanisms and the identification of potential genes involved in host recognition and other common traits. Besides comparative genomics approaches to identify key genes, we have used other genome-guided approaches to search for marker genes of symbiotic interaction to identify symbiotic signals emitted by actinorhizal plant roots. A molecule present in root exudates from *Casuarina glauca* plants induced molecular and physiological changes in *Frankia* including the ability to establish root nodules on the host plant significantly earlier than untreated cells. The presence of an extracellular signaling molecule(s) produced by the exudates-treated *Frankia* was identified by the use of a bioassay with transgenic *C. glauca* plants and specific genetic markers in the nodulation pathway. These results provide support and insight on the hypothesis of chemical signaling between actinorhizal host plant and *Frankia*.

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Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota

JESSICA ANN THOMPSON[1], RITA ALMEIDA OLIVEIRA[1], ANA DJUKOVIC[2], CARLES UBEDA[2,3] AND KARINA DE BIVAR XAVIER[1,4]

[1] *Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal*
[2] *Departamento de Genómica y Salud, Centro Superior de Investigación en Salud Pública, FISABIO, Valencia, Spain*
[3] *Centers of Biomedical Research Network (CIBER) in Epidemiology and Public*

The mammalian gastrointestinal tract harbours a diverse and complex resident bacterial community, which interacts with the host in many beneficial processes required for optimal host health. The ability of bacteria to communicate within and between species through small signalling molecules to regulate behaviours at the community level is well-established. Such communication, known as quorum sensing, might play a role in the homeostasis of the gut microbiota. The quorum sensing signal autoinducer-2 (AI-2) is a strong candidate for this communication because it is produced by many species of the microbiota and it and has been shown to foster interspecies communication. We engineered the enteric bacterium *Escherichia coli* to manipulate the levels of the interspecies quorum sensing signal AI-2 in the mouse intestine and using the established model for *E. coli* colonisation of streptomycin-treated mice, we determined the impact of AI-2 manipulation on antibiotic-induced disruption of the resident microbiota. We showed that AI-2 levels influence the abundance of the major phyla of the gut microbiota, the balance of which is known to impact upon human health.

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Session Chairs: [1]Christian Printzen, [2]Fernando Fernández-Mendoza
Chair affiliations: [1]Senckenberg Research Institute and Natural History Museum, Germany
[2]Germany Karl-Franzens-Universität Graz, Austria

Connecting habitats

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Differential thermal stress responses of scleractinian corals habouring similar ITS2 *Symbiodinium* symbionts

RANJEET BHAGOOLI

Department of Marine & Ocean Science, Fisheries & Mariculture, Faculty of Ocean Studies, University of Mauritius, Réduit 80837, Mauritius

While symbiont change may increase the adaptive potential of the coral holobiont, stress responses of genetically similar ITS2 *Symbiodinium* types harboured by different corals are uncharted. *Symbiodinium* ITS2 types C1 and C21a harboured by *Pachyseris rugosa* and *Stylophora pistillata*, and by *Galaxea fascicularis* and *Favia halicora*, respectively, were exposed in hospite to 28 and 31oC for 4 days and left to recover during 4 days. Photosystem II functioning (Fv/Fm) of C1 isolates declined irrespective of the host but were more susceptible to 31oC over 36 hours exposure than C21a. Fv/Fm responses of in hospite C1 symbionts were depended on their respective hosts. Fv/Fm of in hospite C1 symbionts in *S. pistillata* and *P. rugosa* declined significantly at 31oC after days 1 and 4, respectively, while that of in hospite C21a remained unchanged in both tested hosts. At 31oC, antioxidant defense enzyme activities (SOD and CAT) were higher and sustained in *P. rugosa* intact tissue than in *S. pistillata* and 2-fold higher in *F. halicora* than in *G. fascicularis*. *Symbiodinium* C1 SOD and CAT increased only in *P. rugosa*. Malondialdehyde content (MDA) levels, indicative of oxidative damage, increased at 31oC in intact tissue of *S. pistillata* on day 1 and in *G. fascicularis* on day 2. This preceded the loss of *Symbiodinium* which were photosynthetically affected only in *S. pistillata*. MDA levels for intact *F. halicora* and *P. rugosa* remained unchanged. MDA levels increased only in *Symbiodinium* C1 of *S. pistillata*. Bleaching occurred in *S. pistillata* both via decrease in *Symbiodinium* density and chlorophyll (a+c2) content as from day 2 while in *G. fascicularis* only density declined as from day 3. *P. rugosa* and *F. halicora* did not bleach. These results indicate that genetically similar photosynthetic symbionts inhabiting different coral hosts may lead to variable holobiont thermal stress responses.

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Symbiodinium clade distribution in *Acropora muricata*: implications for physiological performance

R BHAGOOLI[1], S MATTAN-MOORGAWA[1], SB SADALLY[2], PK CHUMUN[2], N TALEB-HOSENKHAN[2]

[1]*Department of Marine & Ocean Science, Fisheries & Mariculture, Faculty of Ocean Studies, University of Mauritius, Réduit 80837, Mauritius*
[2]*Department of Biosciences, Faculty of Science, University of Mauritius, Réduit 80837, Mauritius*

Coral bleaching, through the loss of their symbionts, genus *Symbiodinium*, may act as a window for change of their unicellular photosynthetic symbionts from weak (e.g. clade C) to more stress- robust ones (e.g. clade D). However, the eco-physiological implications of a coral host harbouring symbionts of clade C, D or a combination of C and D are yet to be fully explored. This study investigates the zooxanthellae clade (Dec 2008-Jan 2013), photo-physiology, chlorophyll a (Chl a) concentration, estimated productivity and coral linear extension rate (Dec 2012-Dec2013) in the branching coral *Acropora muricata* from the Flic-en-Flac lagoon, Mauritius. Zooxanthellae clades in *A. muricata* exhibited a changing trend from clade C dominance in December 2008 to clades D and C+D in January 2013. This might be attributed to a severe bleaching event recorded in Jan-Mar 2009. The maximum relative electron transport rate (rETRm), determined in Jan 2013 through rapid light curves using a Diving-PAM, was higher (1.5 fold) in *A. muricata* habouring either clade C or C+D than those having clade D. Chl a concentration per cell for clade C and C+D symbionts were about 1.6 and 1.2 fold higher than the clade D ones. Estimated productivity, calculated by the product of rETRm and chl a, was lowest in *A. muricata* harbouring clade D and highest for clade C. The coral linear extension rate, measured between Jan 2012 to Jan 2013, revealed an almost 2 fold higher growth rate in clade C and C+D than the clade D colonies. These results suggest that colonies of *A. muricata* harbouring clade C or clades C+D exhibit a better photosynthetic and coral growth performance than those with clade D. This implies that harbouring of C+D clades could potentially enhance the lower physiological performance of *A. muricata* colonies containing the thermally more robust clade D.

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Contrasting host and symbiont biogeography in a marine defensive symbiosis

NICOLE B. LOPANIK[1], JONATHAN LINNEMAN[1], DARCY PAULUS[2], GRACE E. LIM-FONG[2]

[1]*Department of Biology, Georgia State University, Atlanta, GA*
[2]*Department of Biology, Randolph-Macon College, Ashland, VA*

The invasive, temperate marine bryozoan *Bugula neritina* possesses an uncultured, vertically-transmitted bacterial symbiont that produces unpalatable natural products, bryostatins, which protect the host larvae from predation. Previous research showed that the host was in fact a complex of closely related sibling species and that in the eastern Pacific, the symbiont varied in the types of bryostatins produced. Interestingly, some host populations (Type N) in the western Atlantic are aposymbiotic, and do not possess the deterrent bryostatins. The aposymbiotic host populations appeared to be restricted to higher latitudes, while defended, symbiotic bryozoans (Type S) were found primarily in lower latitudes. This distribution pattern of host and defensive symbiont seems to reflect biogeographical trends, in which organisms at lower latitudes were subject to higher levels of predation pressure. In surveys of *B. neritina* populations along the western Atlantic, we found Type N individuals at low latitudes, and Type S colonies at higher latitudes, indicating a more widespread host distribution than previously thought. Curiously, some of those host colonies varied in their symbiotic status, with Type N colonies at low latitudes possessing the symbiont whereas Type S colonies collected from high latitudes appeared to be aposymbiotic. Furthermore, the symbiont in Type N colonies at low latitudes appears to be the same strain as that found in Type S. Together, these data indicate that the symbiont, but not the host, is restricted by biogeography, and that symbiont transmission is more flexible than previously thought. As defensive symbiosis seems to be an important trait in marine habitats, understanding factors that affect the distribution of both the host and symbiont are necessary to fully appreciate the ecological impact of symbiosis.

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Presentation type: Poster
Name, Contact: Louis Yohan, yohan.louis1@umail.uom.ac.mu

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Seasonal zooxanthellar clade C photo-physiology in *Acropora muricata* colonies with different light and thermal histories

YOHAN LOUIS[1], SUSHMA MATTAN-MOORGAWA[2], SABRINA D DYALL[1], RANJEET BHAGOOLI[2]

[1] *Department of Biosciences, Faculty of Science, University of Mauritius, Réduit, Mauritius.*
[2] *Department of Marine & Ocean Science, Fisheries & Mariculture, Faculty of Ocean Studies, University of Mauritius, Réduit, Mauritius*

Bleaching of *Acropora muricata* was observed in reef flat (RF) but not in near coast (NC) colonies in Belle Mare, Mauritius. Seasonal fluctuations in environmental data, photo-physiology and zooxanthellae clade composition of *A.muricata* colonies were simultaneously examined to understand the variable bleaching pattern within this coast-reef scale. Sea temperature (ST) and light intensity were recorded using data loggers deployed at NC and RF stations. In situ photo-physiological parameters of zooxanthellae were measured with a Diving-Pulse-Amplitude-Modulated Fluorometer (D-PAM). Zooxanthellae clades were identified by polymerase chain reaction and restriction fragment length polymorphism analysis (PCR-RFLP) of 18S-rDNA. *A.muricata* harboured a *Symbiodinium* clade C variant at both stations. Both stations experienced maximum summer ST of 30.5oC but daily ST fluctuations in summer and winter 2014 were higher in NC station (3-5oC) compared to RF station (1-1.5oC). RF station experienced higher light intensity both in summer (11600 lux/ft2) and winter (5000 lux/ft2). Zooxanthellae had higher effective quantum yield (0.60) at photosystem II (PSII) in winter for both stations. Maximum non-photochemical quenching (NPQm) at PSII was higher in summer for NC colonies but slightly higher for RF colonies compared to winter despite experiencing a 2.5 fold higher light intensity. Maximum relative electron transport rate (rETRm) was higher in summer for NC colonies (120 μmol electrons m^{-2} s^{-1}) but lower for RF colonies (62 μmol electrons m^{-2} s^{-1}) compared to winter (80 μmol electrons m^{-2} s^{-1}). A relatively lower summer NPQm of RF colonies at higher light intensities, may imply lower photo-protection effectiveness compared to NC colonies. Lower rETRm of RF colonies in summer may suggest a dysfunction of the PSII apparatus and an early sign of bleaching. These differences in photo-physiological responses may make RF colonies more susceptible to bleaching compared to NC colonies and thus possibly explain such bleaching variability at a coast-reef scale.

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Insights in the lichen-formig species complex *Tephromela atra*: mycobiont-photobiont specific association defines a new taxon

LORENZO CESTARO[1], MARTIN GRUBE[2], LUCIA MUGGIA[1,2]

[1] *University of Trieste, Department of Life Science, via Giorgiari 10, 34127 Trieste, Italy*
[2] *Karl-Franzens University of Graz, Institute of Plan Science, Holteigasse 6, 8010 Graz, Austria*

Lichens are one of the best examples for understanding how the well-ballanced association between symbionts is coordinated and how symbionts evolution and diversity modell patterns of phenotypic plasticity and geographic distribution. The lichen-forming fungi of the genus *Tephromela* particularly offers this possibility, as they form a complex of species widely distributed worldwide and presenting different degree of specificity towards their photobionts *Trebouxia*. Here we studied in detail a taxon recognised to build a monophyletic lineage within the *Tephromela* atra species-complex and to associate with high specificity with a new *Trebouxia* lineage. This *Tephromela* taxon grows on bark and is geographycally restricted to the Pacific North West coast and interior of North America; it further differs from the other species of the complex by its secondary chemistry. We combined morphological, chemical and molecular characters, ecological preferences and geographic distribution to segregate it from other epiphytic *Tephromela* species, for which we further studied the genetic diversity and the specificity of their associations with *Trebouxia* photobionts.

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Mycobiont-photobiont interactions of the lichen *Cetraria aculeata* in high alpine regions of East Africa and South America

TETIANA LUTSAK[1], FERNANDO FERNÁNDEZ-MENDOZA[2], CHRISTIAN PRINTZEN[1]

[1] *Senckenberg Research Institute and Natural History Museum Frankfurt, Germany*
[2] *Karl-Franzens-Universität Graz, Austria*

Lichens are symbiotic systems of a fungal mycobiont and one or more photosynthetic partners (photobionts). From an ecological viewpoint the lichen symbiosis is extremely successful, as lichens are found in almost all terrestrial habitats from the tropics to polar regions. The symbiotic lifestyle enables the mycobiont and photobiont to colonize many habitats in which they could not exist otherwise. Specific mechanisms of mycobiont-photobiont interactions, for example photobiont switches, may explain the broad ecological niches of many lichens and might also drive speciation. *Cetraria aculeata* is a brown fruticose lichen species frequently found in open polar and boreal environments from the maritime Antarctic to the high Arctic, including high mountain ranges. The species also occurs in forest gaps, steppe and coastal ecosystems of the Mediterranean and temperate zones. Its bipolar, subcosmopolitan range and the wide ecological niche make it particularly useful as a model species to study processes of symbiotic interactions, adaptation and speciation. Based on a worldwide population sample of *C. aculeata* we focus on mycobiont-photobiont interactions in populations from tropical alpine mountain ranges in East Africa and South America. We focus on the questions how the extreme ecological conditions of these mountain ranges affect levels of haplotype diversity in mycobionts and photobionts and whether they induce the association with particular photobionts. We show that unique haplotypes as well as widely distributed polar photobionts can be found in tropical alpine environments and discuss the phytogeographical and ecological implications of these findings.

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Session: Connecting habitats
Presentation type: Poster
Name, Contact: Mattan-Moorgawa Sushma, s.moorgawa@uom.ac.mu

Temperature and light effects on the symbiont photo-physiology, density and clade diversity in the coral *Acropora muricata* from different zones of a tropical reef

PK CHUMUN[1], S MATTAN-MOORGAWA[2], SB SADALLY[1], N TALEB-HOSENKHAN[1], R BHAGOOLI[2]

[1] *Department of Biosciences, Faculty of Science, University of Mauritius, Réduit 80837, Mauritius*
[2] *Department of Marine & Ocean Science, Fisheries & Mariculture, Faculty of Ocean Studies, University of Mauritius, Réduit 80837, Mauritius*

Increase in bleaching incidents has prompted research on the mutualistic symbiosis between corals and zooxanthellae and its importance in adaptation to changing environmental conditions. This study targeted the effect of temperature and light on the photo-physiology of the coral *Acropora muricata* and its association with zooxanthellae clades, at different sampling stations (shore, lagoon and reef) from Belle Mare reef, Mauritius. The coral samples were incubated at different light (200 μmol*m^{-2}s^{-1}and 800 μmol*m^{-2}s^{-1}) and temperature (28 °C and 32 °C) conditions for 3hrs which was followed by a recovery period (21hrs) at 200 μmolm^{-2}s^{-1} and 28 °C. Measurement of the maximum quantum yield (Fv/Fm) after 3hrs stress, by a JUNIOR PAM, indicated that corals collected from reef, incubated at 32 °C and 800 μmol*m^{-2}s^{-1} (R32H), had a significantly lower (~0.5 fold) Fv/Fm than corals collected from shore, incubated in the same condition (S32H). However, zooxanthellae density did not change significantly between these two conditions. During the recovery phase, corals from R32H were not able to recover and the Fv/Fm was below 0.200 compared to S32H that showed a recovery from 0.312 ± 0.051 (n = 5) to 0.456 ± 0.034 (n = 5). In addition, R32H bleached at the end of the experiment compared to S32H, which did not exhibit any bleaching sign. The 18S-rDNA region of the symbiont was amplified and identification of the clades indicated a higher abundance of clade C in corals from reef and lagoon whereas a mixture of clade C+D was more prominent from the shore. This association of a thermally tolerant clade (D) with the generalist clade C may be an adaptation of corals to more stressful conditions (shore). This may be of utmost importance for choosing the adequate region in a reef for conservation and management purposes.

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Variable stress responses of tissue balls from hard corals harbouring clades C and D symbionts

S MATTAN-MOORGAWA[1], SB SADALLY[2], PK CHUMUN[2], N TALEB-HOSSENKHAN[2], SDDV RUGHOOPUTH[3] AND R BHAGOOLI[1]

[1]*Department of Marine & Ocean Science, Fisheries & Mariculture, Faculty of Ocean Studies, University of Mauritius*
[2]*Department of Biosciences, Faculty of Science, University of Mauritius 3Department of Physics, Faculty of Science, University of Mauritius*

This study investigated the effects of thermal stress and extracts of sponge (*Haliclona* sp.) and ascidian (*Didemnum molle*) on the tissue balls (TBs) density from three hard corals and on the photosystem II functioning (effective quantum yield, FϕPSII) of their zooxanthellae. The corals *Fungia repanda* and *Pocillopora damicornis* harboured clade C symbionts while *Acropora muricata* had either clades C or D. Thermal stress experiments, in absence of chemical stress, revealed that higher temperatures, 28 and 31˚C, tended to have a negative effect on the TB density from *A. muricata* with clade C and *F. repanda* but not from *P. damicornis*, when compared to the control temperature of 25˚C. FϕPSII decreased in A. *muricata* with clade C but remained stable in *F. repanda*, *P. damicornis*, and *A. muricata* with clade D, under the effect of thermal stress. A combination of thermal stress with 50μg*ml^{-1} *D. molle* extract, had more pronounced effect on the density of the TBs, but did not affect the FϕPSII in all three species, irrespective of the symbiont clade harboured. An increased concentration of *D. molle* extract at 200μg*ml^{-1} combined with thermal stress, also affected the FϕPSII in *A. muricata* with clade C and *F. repanda*, as compared to *P. damicornis*. Thermal stress with 50μg*ml^{-1} *Haliclona* sp. extract, affected the TB density in all three coral species, but caused a reduced FϕPSII only in A. *muricata*, as compared to the 200μg*ml^{-1} *Haliclona* extract with thermal stress which affected both TB density and FϕPSII in all three corals. The results suggest a variable susceptibility among the three corals, with *A. muricata* harbouring clade C being most susceptible. This implies that under climate change-driven ocean warming the variable chemical stress responses of A. *muricata* harbouring different symbiont clades might interact differently with other sessile marine organisms.

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Session Chairs: J Dick van Elsas
Chair affiliations: University of Groningen, Netherlands

Ecology and functionality of microbiomes

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Species-specific diversity of novel bacterial lineages and differential abundance of pathways for toxic compound degradation in scorpion gut microbiota

LUIS M. BOLAÑOS[1], MÓNICA ROSENBLUETH[1], SANTIAGO CASTILLO-RAMÍREZ[1], ESPERANZA MARTÍNEZ-ROMERO[1]

[1] *Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México*

Scorpions are considered “living fossils” that have conserved ancestral anatomical features and have adapted to numerous habitats. However, their gut microbiota diversity has not been studied. Here, we characterized the gut microbiota of two scorpion species, *Vaejovis smithi* and *Centruroides limpidus*. Scorpion gut microbiota is species-specific and food deprivation reduces bacterial diversity. 16S rRNA gene phylogenetic analysis revealed novel bacterial lineages showing a low level of sequence identity to any known bacteria. Furthermore, these novel bacterial lineages were each one restricted to a scorpion species. We found that the predicted metagenomic profiles revealed a core set of pathways that were highly abundant in both species and mostly related to amino acid, carbohydrate, vitamin and cofactor metabolism. Additionally, comparisons between predicted metagenomic profiles showed that toxic compound degradation pathways were more abundant in *Centruroides limpidus* recently captured scorpions. This study gives a first insight into the scorpion gut microbiota and provides a reference for future studies on the gut microbiota from other arachnid species.

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Microbial community structure and functional attributes of symbionts associated to sponges of the genera *Aplysina* and *Dysidea*

MARYAM CHAIB DE MARES[1], DETMER SIPKEMA[2] AND JAN DIRK VAN ELSAS[1]

[1] *Department of Microbial Ecology, University of Groningen, The Netherlands*
[2] *Laboratory of Microbiology, Wageningen University, The Netherlands*

Sponges harbor complex microbial communities comprising up to thousands of taxa. Therefore, the molecular basis of symbioses between sponges and their associated microbes remains poorly understood. An essential step in this direction is to identify key microbial players in the symbiosis and characterize their functional attributes. We undertook this task for communities of Bacteria, Archaea, and Eukarya in sponge species of the genera *Aplysina* and *Dysidea* in the Caribbean and Mediterranean Sea using a phylogenetic approach. Compared to the seawater in both locations, microbial communities from all three Domains are less diverse but highly specific to their host, as reported previously. In addition, we found sponge phylogeny to be a better predictor of microbial communities than geographic location. Regarding Archaea, *Nitrosopumilus*, a chemolithoautotrophic nitrifier, was highly enriched in sponges of both genera; and *Aplysina* species uniquely selected taxa involved in sulfur metabolism (*Desulfurococcales*) and methanogens (*Methanopyrus*). These results were in agreement with the more anaerobic environment provided by *Aplysina* species to its symbionts. For Bacteria, as reported before, we found higher diversity in *Aplysina* compared to *Dysidea* species. However, observed dominant taxa differ according to the technique: shotgun metagenomics showed Poribacteria as the most dominant player in *Aplysina*, as well as more even communities, while 16S rRNA data showed genera of Actino- and Acidobacteria as most abundant. These observations were highly replicable across individuals for both techniques. In *Dysidea*, key players were *Bacteroidetes*-like and *Chloroflexi*-like taxa. For microeukaryotes preliminary analyses suggested that differences in taxonomic diversity between the two sponge genera also held for this Domain. Additional ongoing work is the functional characterization of bacterial and archaeal key players present in these sponges, using metagenomics and metatranscriptomics. Combining these approaches should help reveal species interdependencies as well as identify potential targets for natural products research.

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Mycorrhizal community of cork oak (*Quercus suber* L.) forests in two different agro-forestry ecosystems

FRANCISCA REIS[1], PAULA BAPTISTA[2], RUI TAVARES[1], TERESA LINO-NETO[1]

[1]*Biosystems & Integrative Sciences Institute (BioISI), Plant Functional Biology Center (CBFP), University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*
[2]*Mountain Research Center (CIMO), Polytechnic Institute of Bragança, School of Agriculture*

Cork oak (*Quercus suber* L.) is an evergreen oak tree, typical from the Western Mediterranean region. Portugal is the world's largest cork producer with 150,000 tonnes (52.5%) harvested each year. Besides their socio-economic importance, cork oak forests present a high biodiversity which is protected by EU (Habitats Directive 92/43/EEC). Cork oaks occur mostly in savannah-type formations (montados) in Portugal´s southern. In this forest type, cork extraction is made each 9 years and tree density is low (usually 50–150 trees/ha). In past years, cork oak plantation has been done in more dense forests (around 400 trees/ha), especially in the north of the country, which are called "sobreirais". This kind of intensive cork production does not allow the practice of agriculture underneath the trees. Microbial community plays an essential role in forest sustainability. Symbiotic microorganisms such as ectomycorrhizal (ECM) fungi confer advantages to trees, promoting water/nutrient supply and diseases resistance. ECM fungal richness on cork oak montados has been correlated with landscape and land use practices. As edaphic, geographic and forest type are completely different between northern and southern Portuguese cork oak forests, the way these factors influence ECM community could give some clues about cork oak sustainability in both regions. Samples from cork oak field trees were collected in 3 different "sobreiral" forests in northern Portugal [Macedo de Cavaleiros, Sobreiral da Ermida (Gerês) and Sobreiral do Rio Cabril (Gerês)] and "montado" forests in southern Portugal [Grândola, Monte Asparão and Herdade da Contenda (Moura)]. Soil samples were analysed by ITS barcoding on ECM tips and metabarcoding using Illumina platform. This evaluation on cork oak ECM communities could give new insights on the role of fungal symbiosis in the ecosystem functioning of both types of cork forest.

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Multitrophic interactions in the sporosphere of the plant beneficial symbiont *Rhizoglomus intraradices*

MONICA AGNOLUCCI, FABIO BATTINI, CATERINA CRISTANI, MANUELA GIOVANNETTI

Department of Agriculture, Food and Environment, University of Pisa

Arbuscular mycorrhizal fungi (AMF, Glomeromycota) establish beneficial associations with the roots of most land plants, including important food crops, from cereals to legumes, vegetables and fruit trees, and contribute to key agroecosystem processes, such as nutrient uptake, soil aggregation and carbon sequestration. In addition, AMF protect plants from soilborne fungal pathogens and abiotic stresses, such as drought and salinity. AMF performance is affected by the diverse bacterial communities strictly associated with their spores, which differentially enhance the availability of nutrients, such as P, N, S, K, Ca, Cu and Zn. In our work we utilized: i) a culture-independent approach, PCR-DGGE, to identify the diverse bacterial species associated with *Rhizoglomus intraradices* IMA6 spores; ii) a culture-dependent approach to isolate such bacteria and detect their functionally important traits. Sequence analysis of the major DGGE bands showed the occurrence of *Arthrobacter*, *Bacillus*, *Herbaspirillum*, *Massilia*, *Pseudomonas*, *Rhizobium*, *Streptomyces* genera, possibly playing functional roles in the enhancement of nutrient availability to fungal mycelium and plant roots, in plant pathogens control and in plant growth promotion. Isolation in pure culture of the microbiota associated with *R. intraradices* spores showed the occurrence of 374 strains, which were functionally characterized for P-solubilizing, chitinase, nitrogen fixing activity and production of siderophores and indole acetic acid (IAA). Phosphatase and phytase activities were detected in 73% and 100% of *Actinobacteria*, in 74% and 83% of chitinolytic bacteria and in 44% and 52% of nitrogen-fixers. Variable combinations of such bacteria may be at the basis of the differential performance of AMF isolates, in terms of infectivity and efficiency. The isolation of bacteria strictly associated with AMF spores and the investigation of their functional significance represent an essential step, in order to select the best AMF/bacteria combinations to be used as biofertilisers and bioenhancers in sustainable agroecosystems.

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The hitchhiker's guide to symbiosis: A plant pathogen as an insect's defensive mutualist

LAURA V. FLÓREZ[1], PAUL GAUBE[1], MARTIN KALTENPOTH[1,2]

[1] *Insect Symbiosis Research Group, Max Planck Institute for Chemical Ecology, Jena, Germany*
[2] *Johannes Gutenberg University Mainz, Institute for Zoology, Department for Evolutionary Ecology, Johann-Joachim-Becher-Weg 13, 55128 Mainz, Germany*

While many of the insect-bacteria symbioses described so far have a nutritional basis, an increasing number of defensive alliances are being discovered. However, to date there is no direct evidence of a bacteria-mediated protective role at the egg stage in an insect. Beetles within the Lagriinae subfamily (Tenebrionidae) live in association with *Burkholderia* symbionts (ß-*Proteobacteria*), a genus which exhibits extraordinary metabolic and ecological versatility. The symbionts are transmitted vertically to the offspring via contamination of the egg surface, which allows for generating symbiont-free beetles via egg-surface sterilization. In vivo assays testing fungal infestation on symbiont-containing and symbiont-free *Lagria villosa* eggs revealed that the presence of *Burkholderia gladioli* on the surface of the eggs significantly inhibited fungal growth, strongly suggesting a defensive function of the symbionts. Furthermore, *in vitro* bioassays confirmed growth inhibition caused by the symbiotic *B. gladioli* against several mold and entomopathogenic fungi, and whole genome sequencing provided promising candidates for bioactive secondary metabolites. Interestingly, first experiments also demonstrate the plausibility of insect-mediated transmission of *Burkholderia* to the beetles' host plants, which may thus serve as reservoirs for the exchange of symbiotic bacteria. Conclusions and significance: The observed egg defense strategy may reveal an unexplored component of symbiotic functions in arthropods, in which metabolites produced by associated bacteria result in effective chemical protection of the nutrient-rich and immobile egg stage. From the microbe's perspective, the advantage of dispersal to plant hosts via the insect might be a major factor promoting the symbiotic association.

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Bacterial community composition and diversity in an ancestral ant fungus symbiosis

KATRIN KELLNER[1], TIMOTHY A. LINKSVAYER[2], ULRICH G. MUELLER[1]

[1] *University of Texas at Austin*
[2] *University of Pennsylvania*

Fungus-farming ants (Hymenoptera: Formicidae, *Attini*) exhibit some of the most complex microbial symbioses because both macroscopic partners (ants and fungus) are associated with a rich community of microorganisms. The ant and fungal microbiomes are thought to serve important beneficial nutritional and defensive roles in these symbioses. While most recent research has investigated the bacterial communities in the higher attines (e.g., the leaf cutter ant genera *Atta* and *Acromyrmex*), which are often associated with antibiotic-producing *Actinobacteria*, very little is known about the microbial communities in basal lineages, labeled as "lower attines", which retain the ancestral traits of smaller and more simple societies. In this study we used 16S-amplicon pyrosequencing to characterize bacterial communities of the lower attine ant *Mycocepurus smithii* among seven sampling sites in central Panama. We discovered that ant and fungus garden associated microbiota were distinct from surrounding soil, but unlike the situation in the derived fungus-gardening ants, which show distinct ant and fungal microbiomes, microbial community structure of the ants and their fungi were similar. Another surprising finding was that the abundance of Actinomycete bacteria was low and instead, these symbioses were characterized by an abundance of *Lactobacillus* and *Pantoea* bacteria. Furthermore, our data indicate that *Lactobacillus* strains are acquired from the environment rather than acquired vertically.

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The honey bee gut microbiome is involved in the breakdown of host dietary macromolecules

FREDRICK J. LEE, JAMES B. MCKINLAY, IRENE L.G. NEWTON

Department of Biology, Indiana University, Bloomington, IN, USA

Previous work by our laboratory utilized next generation sequencing to construct the first metatranscriptome of the honey bee gut microbiome. We identified three major bacterial classes that are active in the gut (γ-*Proteobacteria*, Bacilli and *Actinobacteria*), all of which are predicted to participate in the breakdown of complex macromolecules (e.g. polysaccharides and polypeptides), the fermentation of component parts of these macromolecules, and the generation of various fermentation products, such as short-chain fatty acids and alcohol. Based on the metatranscriptomic data, we hypothesize that the gut microbiome of the honey bee is involved in dietary processing of food consumed by the host. In order to further examine the functional potential of the gut microbiome, we are currently performing several molecular techniques to address the predicted metabolism of the gut community as a whole, as well as, characterizing representative isolates of transcriptionally dominant bacteria. Collectively, the preliminary results from our research corroborate our hypothesis that the gut microflora of the honey bee harbors bacterial members with unique roles, capable of aiding in the processing of the host's plant-derived diet. Future work will focus on determining the importance of the gut microbiome to host health and digestion.

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The cultivation system can influence the physiological functionality and social interaction of phosphate solubilizing bacteria from the rhizosphere of *Carica papaya* L.!

JULIANA MELO[1,2], ALESSANDRO RAMOS[1], CRISTINA CRUZ[1], MANUELA CAROLINO[1], PATRÍCIA CORREIA[1], LUIS CARVALHO[1]

[1] *Dept. Biologia Vegetal, Faculdade de Ciencias de Lisboa, Campo Grande Bloco C-2, Piso 5, Sala 03, 1749-016 Lisboa-Portugal Environmental Microbiology and Biotecnology Lab, Ecophysiology of symbiotic interactions*
[2] *Unit, Universidade Vila Velha (UVV), Boa Vista, Vila Velha, ES, 29102-770, Brazil*

Agricultural practices can affect the soil environment, and consequently modify the dynamics of microbial communities. The soil microbial activity is essential for nutrient cycling processes and plant nutrition. Phosphorus (P) is an essential nutrient for plants, however, in most soils it is out of the way, because it tends to be associated with the oxides of iron, aluminium and calcium. Thus, the use of microorganisms capable of solubilizing phosphate insoluble forms has been gaining momentum in the research. The present study aimed to isolate bacteria in the rhizosphere of *Carica papaya* L., grown in conventional and organic planting systems, assessing their ability to solubilize phosphate and verify that the type of management influence on functional cooperation in phosphate solubilizing and bacterial growth and physiological diversity. Bacteria were isolated from the rhizosphere of *C. papaya*, 5 phosphate solubilizing bacteria (BSF) of conventional crops, and 7 from organic farming system. The ability to solubilize phosphate was initially tested in a solid medium, and subsequently on NBRIP NBRIP liquid medium P to quantify free in the medium. The highest concentrations of P free, or extracellular, were found in medium with growth of bacteria isolated from the organic farming system. A cooperative behaviour in cell growth was observed independently of the origin of the bacteria. However, no cases of physiological cooperation on phosphate solubilization was observed between isolates of the conventional system. While a positive physiological cooperation was found in the interaction between bacteria of the organic system or conventional / organic mixture. The combined use of these bacteria as environmentally sustainable bioinoculantes is a viable alternative to promote the growth of *C. papaya* plants in tropical soils. The physiological cooperation in phosphate solubilization may be extremely important to the understanding of the behaviour of these organisms when used together as bioinoculantes.

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Photosynthetic symbionts of planktonic *Radiolaria*

IAN PROBERT[1], RAFFAELE SIANO[2], JOHAN DECELLE[1], TRISTAN BIARD[1], FABRICE NOT[1]

[1] *UPMC-CNRS Station Biologique de Roscoff, Roscoff, France*
[2] *IFREMER Centre de Brest, Plouzané, France*

Symbiotic associations involving photosynthetic microalgal symbionts are common in both benthic and pelagic ecosystems and are essential for establishing and maintaining the structure of marine communities. Benthic symbioses involve diverse metazoan hosts, most often in symbiosis with the dinoflagellate *Symbiodinium*. Much less is known about the diversity of hosts and symbionts in planktonic ecosystems. *Radiolarian*s are heterotrophic rhizarian protists that are abundant components of marine planktonic communities. Most radiolarians produce mineralized skeletons and many surface-dwelling taxa are known to harbour microalgal symbionts. We review recent advances in the culture-based morpho-molecular characterisation of different microalgal symbionts of various radiolarian hosts and highlight apparent differences in symbiotic strategies between radialorian groups and between pelagic and benthic systems.

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The microbial community within the marine sponge *Hymeniacidon perlevis*: diversity and changes under laboratory conditions

ANA REGUEIRAS[1,2], VITOR VASCONCELOS[1,2]

[1] *Faculty of Sciences, University of Porto, Portugal*
[2] *CIIMAR-UP Interdisciplinary Centre of Marine and Environmental Research, University of Porto*

Marine sponges are known for harbouring diverse symbiotic microorganisms, with mutual benefits both for the host and the partner. These associations evolved millions of years ago, and their study can help to understand the diversity of Proto-Eukaryote symbiosis. Many studies are now focusing also in the ability of sponges and their symbionts to produce secondary metabolites (toxins and compounds with pharmaceutical interest). Translocation of sponges from natural environment to laboratory-controlled conditions can be necessary for several studies, witch may influence the symbiotic community. The aim of this study is to assess the microbial community in the marine sponge *Hymeniacidon perlevis*, a common intertidal marine sponge of the Portuguese coast, and to understand if it is affected by laboratory conditions. A 454-pyrosequencing analysis was conducted both from sponge in natural environment and under laboratory conditions. Results show significant changes in the microbial community of *H. perlevis*, being *Proteobacteria* the major phylum present in this sponge. Some organisms, such as Cyanobacteria started growing in the new environment and others almost disappeared. Disturbing the balance that exists between sponge and symbiotic microorganisms can affect both the sponge and the hosts, changing the production of secondary metabolites or even conducting to the animal death. This work shows the need to study the community and its balance prior to conduct more extensive studies.

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SGDB: Symbiotic Genomes DataBase for the integration and access to knowledge on host-symbiont relationships

MARIANA REYES-PRIETO, CARLOS VARGAS-CHÁVEZ, AMPARO LATORRE, ANDRÉS MOYA

University of Valencia, Cavanilles Institute of Biodiversity and Evolutionary Biology

Symbiotic relationships occur throughout the tree of life, either in a commensal, mutualistic or pathogenic manner. The genomes of multiple organisms involved in symbiosis are rapidly being sequenced and becoming available, especially those from the microbial world. Currently there are numerous databases that offer information on specific organisms or models, but none offer a global understanding on relationships between organisms, their interactions and capabilities within their niche, as well as their role as part of a system, in this case, their role in symbiosis. We have developed the Symbiotic Genomes Data Base (SGDB; available at http://symbiogenomesdb.uv.es) as a community database resource for laboratories which intend to research and use information of the genetics and the genomics of organisms involved in these relationships. The ultimate goal of SGDB is to host and support the growing and vast symbiotic-host relationship information, to uncover the genetic basis of such associations. SGDB maintains a comprehensive catalog of completely sequenced and finished genomes of symbionts from hosts throughout the Tree of Life, as well as their genomic features. The catalog was generated using computational tools, custom R scripts, and manual integration of data available in public literature. As a highly curated and comprehensive systems database, SGDB provides web access to all the sequenced and annotated genomes of symbiotic organisms, their features, including genomic sequences and metrics, as well as links to central databases such as NCBI. Three different tools are found within the database to explore symbiosis related organisms, their genes and genomes. Also, an orthology search is available and every table, graph and output is downloadable and easy to parse for further analysis. The robust SGDB will be constantly updated to cope with all the data being generated and included in major databases, in order to serve as an important, useful and timesaving tool.

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Session: Ecology and functionality of microbiomes
Presentation type: Keynote
Name, Contact: Joana F Salles, j.falcao.salles@rug.nl

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The microbiome of bird eggs

JOANA F SALLES[1], B. IRENE TIELEMAN[2], STEPHANIE GRIZARD[1,2]

[1] *Genomic Research in Ecology and Evolution in Nature*
[2] *Behavioural and Physiological Ecology, Groningen Institute for Evolutionary Life Sciences, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands*

The impact that body-associated microbes have in shaping phenotypic variation and physiological flexibility in wild birds is largely unknown. Using eggs as a simplified system, we take an eco-immunological approach to address the link between bacterial communities on eggshells (16S rRNA gene sequencing) and antimicrobials in egg albumen (lysozyme, ovotransferrin and pH), across a range of environments. We focused on the lark family (Alaudidae), known to have a cosmopolitan distribution while sharing similar ecological traits (ground-nesting, incubation pattern, diet). As microbial communities are constrained by environmental and ecological factors, we explored the eggshell microbiome among temperate and tropical zones (Netherlands and Kenya), as well as among distinct climatic habitats in Kenya. The latter revealed higher bacterial abundance in the cool/wet location but higher diversity in the warm ones. Remarkably, expanding this comparison to three other lark species revealed that the eggshell microbiome was similar across large geographic range (>60% shared OTUs). Moreover, the eggshell microbiomes within a few days of laying were most reflective of feather microbiomes of the females incubating the eggs rather than of their cloaca or of the nest lining, highlighting the dominance of horizontal upon vertical microbiome transmission. Finally, analyses along a continuum of incubation revealed eggshell microbiome to be highly dynamic, with both bacterial abundances and diversity increasing at the onset of incubation. Despite the low variation in immune properties in general, we observed a few correlations between antimicrobials and bacterial communities within habitats. In an evolutionary context, we speculate that the low disease risk, as indicated by the lack of bacterial pathogens, did not exert a strong selective force in shaping bird immune response and that the natural eggshell microbiome might provide enough protection (by competitive exclusion), therefore allowing birds to invest less in the otherwise costly immune system.

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Is the output of the plant mycorrhizal symbiosis determined by the plant and fungal symbionts, or by the microbiome?

ANA SOARES[1], ANA CORREIA[1], PATRÍCIA CORREIA[1], LUIS CARVALHO[1], ANA REIS[2], MANUELA CAROLINO[1], CRISTINA CRUZ[1]

[1] *Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa. Campo Grande, 1749-016 Lisboa, Portugal.*
[2] *Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa. Campo Grande, 1749-016 Lisboa, Portugal.*

The rhizosphere has an extremely complex and diverse microbiome which performs a dynamic and intrinsic network of interactions. It is usually assumed that the establishment of endomycorrhizal symbiosis is not specific, but that the outputs of the symbiosis are. In this work we assess the hypothesis of the involvement of the rhizospheric microbiome in the determination of the mycorrhizal benefits to the plant (accessed as biomass accumulation). Maize (*Zea mays*) plants were grown in pots with soil collected from fields used to grow maize and were inoculated with distinct arbuscular mycorrhizal fungi (AMF; *Glomus intraradices*, *Glomus mosseae*, *Glomus claroideum*, *Gigaspora* sp.). At the end of the experiment plants were collected and harvested for their biomass; and the bacterial functional groups (nitrogen fixers, phosphorus solubilizers and *Actinobacteria*) associated with the rhizosphere were assessed through their function, number of colony forming units (CFU), and composition (molecular identification-*Actinobacteria*).Results showed that plant biomass accumulation was dependent on the fungal partner; and that the CFU of each bacterial functional group was dependent on the fungal partner in symbiosis with the plant. A more detailed analysis of the rhizosphere showed that the *Actinobacteria* associated with each of rhizosphere was distinct (in number of CFU, composition, and production of anti-microbial compounds). Our results highlight the importance of the microbial community associated with the rhizosphere in the determination of the mycorrhizal outputs for the plant partner and open the door to the hypothesis of the mycorrhizal benefits to plants being dependent on the presence of key microbial organisms (*Actinobacteria*) in the rhizosphere. Results are discussed in face: 1) to what is known about the importance on the provenience of AMF inoculants; and 2) on their implications on the strategies used to engineer the microbial composition of the rhizosphere.

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Identification of stable and beneficial *Drosophila melanogaster* gut microbiota

INÊS SILVA PAIS[1], MARTA SPORNIAK[1], LUIS TEIXEIRA[1]

Instituto Gulbenkian de Ciência

The microbial community associated with multicellular organisms can strongly influence their host physiology, including nutrition, development and immunity. *Drosophila melanogaster* is an established model organism to study host-microbe interactions. This has been recently extended to the analysis of *D. melanogaster* interaction with the gut-associated microbial community. A small number of bacteria are present in the gut of *D. melanogaster* and bacteria associated with *Drosophila* also impact their physiology. However, there is no bacterium that has been shown to grow in the gut of *D. melanogaster* and to stably colonize it. On the contrary, there is evidence that lab cultures of *D. melanogaster* are associated with bacteria that grow on the fly food and are only transiently in the gut of the flies. We developed a protocol to discriminate between transient and resident gut bacteria. By analysing wild caught *D. melanogaster* we identified bacteria that are stably associated with *D. melanogaster* and proliferate in the gut. We also show that these bacteria are beneficial to the host by improving its developmental time and number of progeny in comparison to axenic flies. This association can, therefore, be ecologically relevant since the adult *D. melanogaster* stably carry beneficial bacteria. Our results also establish a new system to study host-gut microbiota interaction using *D. melanogaster*.

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Do *Bacillus* endophytes and epiphytes of food plants colonize the human digestive tract?

MS TORRES[1], B WATERS[2], M VON TURKOVICH, M[2], DJ TAATJES[2], MA SOARES[3], JE CRAIGHEAD[4], JF WHITE[1]

[1] *Department of Plant Biology and Pathology, Rutgers University, New Brunswick, New Jersey USA*
[2] *Department of Pathology and Laboratory Medicine. University of Vermont College of Medicine, 89 Beaumont Avenue, Burlington, Vermont, USA;*
[3] *Department of Botany*

The human intestinal tract is colonized by a myriad of microbes. How they come to inhabit humans is not well understood. In this research, we analyze a subset of probiotic microbes in genus *Bacillus* to evaluate the possibility that they may move from plant hosts to human hosts after consumption of food plants. It is becoming increasingly clear that species of *Bacillus* are common endophytes and epiphytes of plants, and are also known to be present in the human intestinal tract. The presence of *Bacillus* species in the human intestinal tract was evaluated through isolation of *Bacillus* from mucosa derived from intestinal recessions from 20 males and females ranging from 16 to 88 years. To isolate *Bacillus* from intestinal samples, tissues were stored in 40 alcohol, first heated to 60°C for a 2-hour period, then plated onto 10% trypticase soy agar (TSA). Isolates referable to the *B. subtilis* group were recovered from 18 of 20 of the samples. Identification was made using 16S sequence data. Transmission electron microscopy (TEM) and immunohistochemistry were employed to localize endospores and vegetative cells within tissues. Endospores were located in 5 of 20 of the samples within mucosal lining cells (4) and within the intestinal lumen (1); vegetative cells were identified within the intestinal lumen of 1 sample using immunohistochemistry. To evaluate whether close strains of *Bacillus* are present in food plants, we sampled commercially available potatoes, carrots, radishes, parsnips, sweet potatoes and beets, in addition to some non-cultivated plants. Processing involved heat treatment followed by plating on 10% TSA. Several *Bacillus* spp. were isolated from plant roots. Sequence comparisons between human and plant root isolates using multiple genes indicate that human and plant isolates are closely related strains. Consumption of microbes in plants may be one way the human gut microbiome becomes established.

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Session: Ecology and functionality of microbiomes
Presentation type: Oral
Name, Contact: Sergios-Orestis Kolokotronis, skolokotronis@fordham.edu

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Bacterial communities of blacklegged ticks (*Ixodes scapularis*) across developmental stages

CHRISTINE P ZOLNIK[1], ROBERT J PRILL[2], RICHARD C FALCO[3], THOMAS J DANIELS[1], SERGIOS-ORESTIS KOLOKOTRONIS[1]

[1] *Department of Biological Sciences, Fordham University, Bronx, NY.*
[2] *Healthcare Informatics, IBM Almaden Research Center, San Jose, CA.*
[3] *New York State Department of Health, Armonk, NY*

Blacklegged ticks (*Ixodes scapularis*) are the most important arthropod vector in the United States. Although responsible for transmitting a wide range of pathogens, little is known regarding the overall microbial community found within these ticks. Microbial relationships within ticks may influence their survival, distribution, and ability to maintain and transmit pathogens. However, the lack of knowledge of microbial taxonomy in most organisms hinders our understanding of microbial community composition, diversity, and function. We determined the bacterial species diversity within all three developmental stages of *I. scapularis* collected from the field in southern New York State, where this species is prevalent and Lyme disease incidence is high. Additionally, newly hatched, lab-reared larvae were tested to determine the baseline microbial community structure. 16S rRNA gene amplicon sequencing was carried out on the MiSeq (300bp PE) Illumina platform. OTUs were clustered de novo using a 97% identity threshold and identified with the Greengenes database. *Rickettsia* was the most abundant shared genus across all life stages. Pools of females and lab-reared larvae displayed the lowest bacterial diversity, with 99.5% and 97.8% of OTUs identified as *Rickettsia* spp. respectively. The reduction in bacterial community diversity in the adult female stage is a novel finding and the similarity in bacterial communities in lab-reared larvae and females implies transovarial transmission. We identified six bacterial genera found in all developmental stages representing a core microbiome. Most of these bacteria belong to the *Proteobacteria*, and, with the exception of *Rickettsia*, have species associated with soil, plants, and water habitats, thus suggesting environmental acquisition. Our results on microbial community structure and diversity provide a baseline for future studies that will allow us to identify and assess complex ecological and functional interactions between these microorganisms and their host environment, and open prospects for vector control through targeted biodiversity manipulation.

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Mechanisms used by *Burkholderia terrae* to interact with soil fungi

JAN DIRK VAN ELSAS, IRSHAD UL HAQ, PU YANG

University of Groningen, Netherlands

Burkholderia terrae strains BS001 and BS110 are key bacteria that are interactive with *Lyophyllum* sp. strain Karsten. Strain BS001 produces a biofilm on the latter host and can sequester glycerol from it. Remarkably, strain BS001 can « castrate » its host, significantly inhibiting primordium setting (sporulation) by it. Furthermore, it has broad comigration capacity with fungi through soil, also forming a biofilm on *Trichoderma harzianum*. Comparative analysis of the 11.5 Mb BS001 genome showed numerous genes for putative mycosphere-relevant functions. Interestingly, numerous regions of genomic plasticity (RGP) were found, including a 70,422 kb long region (denoted RGP79, containing a type 4 secretion system (T4SS) and other plasmid-type traits. Furthermore, key biofilm formation genes and secretion systems of types 2, 3 and 6 were present. The type three secretion system was found to have relevance for the interaction with the fungus. The presence of multitude of predicted metabolic pathways and transporters suggests that *B. terrae* BS001 can take up an extreme range of compounds (such as glycerol, methylglyoxal, fatty acids, sugars and amino acids). The genome further contained genes for production of insect toxins as well as degradation of complex compounds. Moreover, clusters encoding nonribosomal peptide synthetases (NRPS) and hybrid polyketide synthases/NRPS were found. The *B. terrae* strain BS001 genome may have acted as a «collector» of habitat-relevant traits, being such habitats bulk soil, fungi and plants. Transcriptome analysis on soil extract agar plates further revealed that strain BS001 modulates the expression of key genetic circuits as a response to both a soil–mimicking environment and fungal hyphae. The stationary–phase sigma factor RpoS, as well as numerous genes under its control, were expressed to a large extent across the treatments over the whole temporal regime. Strain BS001 perceived the presence of fungal hyphae at a distance of 15 mm. Then, chemotaxis–related genes were upregulated. Later, a gene encoding a SET domain containing protein (a potential type 3 secretion system secreted effector protein) was also upregulated. Finally, several genes, including five potentially involved in oxidative stress responses, were highly upregulated by the fungus. Thus strain BS001, being in a stress–dominated status on soil extract agar, showed an early and dynamic response to hyphae of *Lyophyllum* sp. strain Karsten, characterized by dynamically–changing chemotaxis, metabolic signalling and a response to oxidative stress.

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Session Chairs: Luis Vicente Lopez-Llorca
Chair affiliations: Universidad de Alicante, Spain

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Bacterial community associated to *Monochamus*, the insect-vector of pine wilt disease

MARTA ALVES[1,2], PATRÍCIA MATOS[2], ANABELA PEREIRA[2], CLÁUDIA VICENTE[3,4], JOANA HENRIQUES[5], HELENA LOPES[2], MANUEL MOTA[3], ANTÓNIO CORREIA[2], ISABEL HENRIQUES[1]

[1] *Dep. of Biology & iBiMED, Univ. of Aveiro, Portugal*
[2] *Dep. of Biology & CESAM, Univ. of Aveiro, Portugal*
[3] *ICAAM, Dep. of Biology, Univ. of Évora, Portugal*
[4] *Environmental Biology Dep., Chubu Univ., Japan5INIAV, Oeiras, Portugal.*

Wood-boring beetles of the genus *Monochamus* are the dispersing vectors of *Bursaphelenchus xylophilus*, the causing agent of pine wilt disease (PWD). This disease inflicts concerning damages in Eurasian pine forests. Studies in vectoring efficiency for other diseases demonstrate a large influence of symbiotic microorganisms in insects' survival and developmental time. The bacterial community associated to *Monochamus* may also influence the beetle's life cycle, and consequently the development of PWD.The aim of this study was to characterize the bacterial community associated to PWD vectors in Europe and East Asia using a culture-independent approach. For this purpose, *Monochamus galloprovincialis* and *Monochamus alternatus* were collected during the spring of 2013 and 2014 from four different regions in Portugal and from Marioka in Japan. Total DNA was extracted from insect's tracheal system and the presence of *B. xylophilus* was inspected by PCR. The V3 region of the 16S rRNA gene was amplified and analysed using denaturing gradient gel electrophoresis (DGGE). Samples of each location were selected for barcode pyrosequencing.In both methods samples clustered according to host species with significant differences in bacterial composition ($p<0.05$). This might be explained by the presence of some families in *M. alternatus* that are absent in *M. galloprovincialis* (e.g. *Leuconostocaceae* and *Acidobacteriaceae*). However, *Enterobacteriaceae*, *Pseudomonadaceae* and *Rhodobacteriaceae* seem to be transversal to the genus *Monochamus* being *Enterobacteriaceae* the dominant family in both species. These results suggest that different *Monochamus* species harbor different bacterial communities which may influence *B. xylophilus* associated bacteria. The dominant family, *Enterobacteriaceae*, has also been described in association with *B. xylophilus* which indicates that it might be an important family to PWD.The significance of this study relies in the possibility of searching new outlooks to PWD control with the description of the bacterial community associated to pine wilt nematode insect vectors.

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Fluorescent pseudomonads as efficient plant growth promoting and biocontrol agents for diverse crops under saline conditions

NAVEEN K. ARORA, SAKSHI TEWARI

Department of Environmental Microbiology, BBA University, Lucknow - 226025, UP, India

Multi-stress tolerating strains of fluorescent pseudomonads were isolated from the rhizosphere of diverse plants growing in the semiarid regions of west Kanpur, Uttar Pradesh, India. Strains were monitored for their biocontrol and plant growth promoting (PGP) traits under saline and non-saline conditions. Strain Eki and PF23 were selected from the collection as they displayed maximum survivability at saline, temperature and pH stress. Both the strains were able to tolerate high temperature (up to 50°C), salinity (up to 2000 mM NaCl) and noticeable growth over wide range of pH. Eki and PF23 displayed strong antagonism against dreadful phytopathogen of the region, *Macrophomina phaseolina.* Strains also produced diverse PGP and biocontrol metabolites including IAA, siderophore, pyocyanin and exopolysacharides upto 500 mM NaCl. In vivo pot study and field trials were conducted in semi-arid regions (soil having EC 12 dS/m) naturally infested with *M. phaseolina* (103 CFU/g soil) taking chickpea (*Cicer arietinum*) and sunflower (*Helianthus annuus*) as test crops. Eki suppressed charcoal rot disease incidence in chickpea by 62.65% and 53.45% under non-saline and saline conditions, whereas, PF23 suppressed disease incidence by 71% and 63 % in sunflower under non-saline and saline conditions. Thus on the commercial scale, application of these strains can be beneficial and could be a sound step towards sustainable crop production and reclamation of saline lands. Bioformulations developed from these multifaceted strains may be considered as commercially important for renovation of stressed sites, enhancing plant growth parameters and management of charcoal rot disease incidence in diverse crops.

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Fungal endophytes: a strategy for mitigating salt and drought stress on plant growth

KUMKUM AZAD[1], SUSAN KAMINSKYJ[1], JAMES BASINGER[2]

[1] *Department of Biology,*
[2] *Department of Geological Sciences, University of Saskatchewan, Saskatoon, SK, Canada*

Drought and soil salinization are critical abiotic stressors for plant growth that affect crop yield worldwide, particularly through catastrophic weather events and prolonged irrigation practices. Some fungi grow symbiotically inside plants as endophytes, and specifically Class 2 fungal endophytes include strains that may promote plant growth and survival under abiotic stress like salt or drought. Strains of Class 2 endophytes isolated from plants growing in natural highly saline sites (saline lake shores) and human-impacted saline sites (potash tailings areas) in Saskatchewan were used to colonize axenic tomato seeds. Seedlings were grown in double-decker Magenta boxes, so that water quantity and salinity could be controlled precisely to assess endophyte-related plant growth performance under salt and drought stress. Endophyte-colonized plants had higher root (20-50%) and shoot (10-20%) biomass, following 10-15 days salt stress (300 mM, 400 mM, and 500 mM NaCl) treatments than non-colonized plants. This indicated a fitness benefit due to endophyte colonization. Plants were also assessed for drought tolerance by depriving plants of water for 10-11 days. Endophyte-colonized plants had 30-40 % increased root and 10-20 % increased shoot biomass compared to control plants, consistent with 10-25 % better water use efficiency due to colonization. Increased photosynthetic efficacy (10-20 % in 11 d drought stress, and 20-40% in 500 mM NaCl stress) and decreased reactive oxygen species generation (20-40 % in both salt and drought stress) in endophyte-colonized plants were also correlated to endophyte-conferred abiotic stress tolerance. These findings indicate that class 2 endophytes isolated from plants naturally growing on saline soils have high potential to improve agriculture on dry or saline soils.

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Azospirillum brasilense beyond the rhizosphere

ELIZABETH CZERWINSKI, CARL BAUER

Indiana University

Azospirillum brasilense is a plant growth promoting bacterium (PGPB) used in bio-fertilizers world-wide. Plants associated with *A. brasilense* display increased root length, root branching, and root hair formation. Stimulated root development is thought to account for the subsequent increases in plant health and crop production. Thus, *A. brasilense* is traditionally considered to be a rhizosphere mutualist only. Using quantitative PCR and confocal microscopy we show evidence that *A. brasilense* is not confined to the rhizosphere, but associates with the entire plant. This information opens up novel discussion on the precise interactions between *A. brasilense* and its' plant host.

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Effects of the nematophagous fungus *Pochonia chlamydosporia* on the development of tomato and *Arabidopsis*

E A ZAVALA-GONZÁLEZ[1,2], E RODRÍGUEZ-CAZORLA[3], N ESCUDERO[1], A ARANDA-MARTÍNEZ[1], A MARTÍNEZ-LABORDA[3], M RAMÍREZ-LEPE[2], A VERA[3], LV LOPEZ-LLORCA[1]

[1] *Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) "Ramon Margalef", University of Alicante, Apdo. 99, 03080 Alicante, Spain*
[2] *Food Research and Development Unit (UNIDA), Laboratory of Genetics, Technological Institute of Veracruz, 91897 Veracruz, México*
[3] *Area of genetics, Miguel Hernandez University, Campus San Juan, 03550- Alicante, Spain*

Pochonia chlamydosporia is a nematophagous fungus present worldwide in soils infecting eggs of plant parasitic nematodes. *P. chlamydosporia* can also colonize roots of crops such as tomato, wheat and barley promoting their growth. Our results showed that some *P. chlamydosporia* isolates can promote root growth of tomato seedlings and plantlets. *P. chlamydosporia* also reduced flowering and fruiting times of tomato plants in the greenhouse increasing root weight and no. mature fruits/ plant. In *A. thaliana*, *P. chlamydosporia* increased ten fold plant fresh weight and also promoted flowering. Flowering time in flk-2 and ft-10 late-flowering mutants, was reduced by 7 days in comparison to uninoculated control plants. *P. chlamydosporia* increased ca. 6 fold silique (=fruit) production respect to uninoculated control plants. Growth promoting effects may be related with the increase of the expression of FT and SOC1 flowering genes and LOX3 and AOC3 jasmonate synthesis genes, which together regulate plant development.

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Interactomics, plant growth promotion and biocontrol

L. V. LOPEZ-LLORCA

Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) “Ramon Margalef”, University of Alicante, Apdo. 99, 03080 Alicante, Spain

Interactomics, the use of -omics derived technologies to understand organismic interactions, has a large potential in the use of biological inoculants such as Nematophagous (NF) and Entomopathogenic fungi (EF) for managing abiotic and biotic stress in plants, including crops. Examples will be given on the use of Genomics, Proteomics and Metabolomics to understand the biofertilizer and biocontrol capacities of NF and EF. Unlike pathogens, NF and EF are compatible with chitosan, a deacetylated form of chitin, which is a key structural component of barriers of their nematode and insect hosts. Chitosan enhances expression of NF pathogenic determinants such as serine proteases. Interactomics will help in future studies to understand and perhaps modulate gene functions to improve the plant growth promotion and biocontrol capabilities of NF (e.g. *P. chlamydosporia*) and EF which are phylogenetically related.

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Analysis of the tritrophic interaction: Tomato, *Meloidogyne javanica* and *Pochonia chlamydosporia*

N. ESCUDERO

Laboratory of Plant Pathology, Department of Marine Sciences and Applied Biology, Multidisciplinary Institute for Environmental Studies (MIES) "Ramon Margalef", University of Alicante, Apdo. 99, 03080 Alicante, Spain

Pochonia chlamydosporia (Goddard) Zare and Gams (Pc) is a fungal biocontrol agent of cyst and root-knot nematodes, infecting their females and eggs. The fungus is also an endophyte in barley and tomato roots. Pc genome has expanded protease families such as S8 (serine proteases) and S10 (serine carboxypeptidases). SCP1, a newly discovered Pc serine carboxypeptidase, was found in root endophytism. ELISA and immunolocalization detected the production of both VCP1 and SCP1 by Pc infecting nematode eggs. We have studied the tritrophic (root-nematode-nematophagous fungus (Pc)) interaction using a combined chemometrics-metabolomics approach [excitation–emission matrix (EEM) fluorescence spectroscopy, nuclear magnetic resonance (NMR) and high performance liquid chromatography–mass spectrometry (HPLC–MS)]. The analysis of the rhizodeposition of this tritrophic system gives clues for new biocontrol strategies by blocking nematode-plant communication or enhancing plant defences to nematodes.

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A novel biocontrol strategy against root-knot nematodes - exploring the interaction *Pochonia chlamydosporia*-plant defence mechanisms

MARIA CLARA VIEIRA DOS SANTOS[1], ROSANE CURTIS[2], ISABEL ABRANTES[1]

[1] *Centre for Functional Ecology-CFE, department of Life Sciences, University of Coimbra, Calçada Martim de Freitas 3000-456 Coimbra, Portugal*
[2] *Bionemax UK Ltd, Research & Innovation Campus, Rothamsted, Herts AL52JQ, UK*

The sustainable management of economically important pests, such as root-knot nematodes (RKN), *Meloidogyne* spp., calls for the integration of ecologically sound strategies. These include the regulatory effects of nematode natural enemies and activation of natural plant defence mechanisms. The biocontrol agent, *Pochonia chlamydosporia*, is an endophytic facultative egg parasite of cyst nematodes and RKN and can saprophytically proliferate in the rhizosphere using nutrients present in root exudates released in the rhizosphere. However, this fungus is not able to prevent the initial infection of roots by RKN infective juveniles. In previous studies, the use of foliar sprays of cis-jasmone seemed to indirectly increase the biocontrol efficiency of a Portuguese *P. chlamydosporia* isolate against RKN. Activation of specific biochemical defence pathways might produce changes in the plant and/or the rhizosphere that prompt the fungal transition from the saprophytic to the parasitic trophic phase. This research aims to develop a biocontrol strategy that builds on the modulation of plant biochemical pathways and their effects on interacting organisms in the rhizosphere, and exploits such mechanisms to control RKN. Bioassays will be conducted to elucidate the effect of root exudation patterns, in both natural and artificially-activated plant defence, in promoting fungal rhizosphere colonisation and the switch to parasitism in plants treated with various plant activators. To unveil the metabolic pathways of this combined strategy, studies of gene expression in selected specific conditions will be performed. The putative modulation of the interactions amongst the host plant, nematode and fungus when natural defence pathways are activated not only poses new questions on how these organisms interact with the host plant but, furthermore, may unravel a potential new application for the biomanagement of these phyto-endoparasitic nematodes.

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A bacterial endophyte that enhances heat stress tolerance and insect feeding deterrence in ornamental *Hosta* cultivar

HAIYAN LI[1], MARCOS A. SOARES[2], MARIUSZ TADYCH[3], MÓNICA S. TORRES[3], MARSHALL BERGEN[3], JAMES F. WHITE

[1] *Department of Bioengineering, Kunming University of Science and Technology, China*
[2] *Department of Botany and Ecology, Universidade Federal de Mato Grosso, Brazil*
[3] *Department of Plant Biology and Pathology, Rutgers University, USA.*

Seeds of *Hosta* cultivars (August Moon, Blue Umbrella, Halcyon) were screened for endophytic bacteria by first surface-disinfecting by sequentially dipping into 70% ethanol (2 min) and 0.5% sodium hypochlorite (2 min) then plating onto 10% Trypticase Soy Agar (TSA). Several cultivars (Blue Tooth, Delana's Smile, Tongue Twister and Winter Snow) were screened through isolation from petiole tissues disinfected as above. Bacteria were obtained in high frequency from August Moon, Blue Umbrella, Halcyon and Winter Snow. All isolates were identified as *Bacillus amyloliquefaciens* using 16s rDNA sequence data. One strain (Blu-v2) was inoculated into plantlets of cultivar 'Rainforest Sunrise' by spraying a bacterial suspension onto surfaces of plants (4X over 60 days); colonization was confirmed by re-isolation from inoculated plants (endophyte-infected, E+), and failure to isolate from controls (endophyte-free, E-). To test for heat stress tolerance, E+ and E- plants were placed in a growth chamber at 40 °C (70% relative humidity and photoperiod of 15h at 300μE/m2/sec). After 3 days, there was a significant difference ($P<0.05$; t-test) in the average wilting severity between E+ and E- plants in the number of leaves wilting and necrotic, E+ = 8 ± 1.58 (mean ± sd); E- = 17.4 ± 3.21. To assess insect feeding deterrence the leaves of E+ plants and E- plants were excised and used in a 7-day feeding experiment employing the 4th-instar larvae of fall armyworms (*Spodoptera fruigiperda*). We found a significant decrease in weights of larvae fed E+ compared to E- leaves ($P<0.05$; t-test; E- average weight increase = 50.1±20.6 mg and E+ average weight increase = 35.61±12.6 mg), and the death rates of larvae fed E+ leaves was higher compared to that of larvae fed E- leaves (E- % dead = 3.33 and E+ % dead = 30).

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Chair affiliations: Max Planck Institute for Chemical Ecology, Germany

Establishment and maintenance of mutualism

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Insights into evolutionary and immune processes paralleling endosymbiosis establishment and maintenance

ABDELAZIZ HEDDI

University of Lyon, INRA, INSA-Lyon, UMR203 BF2I, Biologie Fonctionnelle, Insectes et Interactions

Interspecific associations are widespread in nature and occur at different levels of organism complexity. In insects, most species thriving on nutritionally poor and unbalanced habitats house integrated intracellular bacteria (endosymbionts) that are transmitted vertically for million years and supplement the host diet with several metabolic products. However, maintaining the beneficial nature of this long-term relationship constrains both the host and the symbiont to adaptive changes in their interactions. For instance, comparative genomics have shown that endosymbionts experience drastic genomic deletions and gene mutations, some of which impact bacterial virulence and their ability to be tolerated by the host. In *Sitophilus-Sodalis* association, we recently have demonstrated that genomic shrinkage occurs rapidly at early stages of symbiogenesis. An extensive IS element expansion was unraveled in the bacterial genome, which appears to have mediated numerous genome rearrangements, deletions, and duplications that might be beneficial to the association.On the host side, the immune system is permanently struggling to keep beneficial symbionts while activating defense effectors to prevent pathogenic infections. We showed that cereal weevils have selected a “compartmentalization” strategy that consists to seclude endosymbionts within the bacteriome organ and to protect them from the host systemic immune response. The bacteriome organ expresses selected immune effectors, including the Coleoptericin A antimicrobial peptide, which targets endosymbionts, inhibits their cell division, and prevents their externalization from the bacteriome. Recently, we showed that endosymbiont load is under the control of a fine-tuned integration of symbiont costs and benefits, according to host developmental and physiological requirements. Weevil endosymbionts intensively multiply in young adults to support a massive need for tyrosine and phenylalanine required during insect cuticle tanning and hardening. Once the cuticle is achieved, endosymbionts are rapidly recycled through apoptosis and autophagy in a tissue-dependent manner that preserves female germline-associated endosymbionts and thus their transmission to next generations.

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A sensor histidine kinase regulates biofilm formation, motility, and cyclic-di-GMP in the symbiont, *Vibrio fischeri*

JOHN F. BROOKS, AND MARK J. MANDEL

Northwestern University Feinberg School of Medicine, Department of Microbiology-Immunology, Chicago, IL

Animals establish and maintain beneficial associations with select environmental bacteria. The molecular mechanisms that allow select environmental bacteria to form beneficial associations with animals are poorly understood. To address this question, we study acquisition of luminous Gram-negative bacteria *Vibrio fischeri*, the exclusive light-organ resident, by the Hawaiian bobtail squid, *Euprymna scolopes*. Here we describe the characterization of a novel bacterial colonization factor that was identified using the INSeq (insertional sequencing) approach in which we apply deep sequencing to enumerate high-density transposon pools before and after host colonization. We examined the behavior of over 40,000 mutants during colonization in 1,500 gnotobiotic squid hatchlings. We focused our analysis on the sensor histdine kinases that were enriched in the hatchlings to uncover signaling pathways important for host colonization. Validation in 1x1 competitions with the parent strain revealed a colonization advantage for the uncharacterized sensor histidine kinase VF_A0360.*V. fischeri* forms biofilm-like aggregates in the squid mucus during the first three hours of colonization, and aggregation is necessary for robust colonization. The VF_A0360 mutant was shown to produce biofilm aggregates 5X larger than the parent strain in the host environment, suggesting a role for VF_A0360 in biofilm regulation. Additionally, the VF_A0360 mutant exhibited a motility defect, suggesting a role for VF_A0360 during free-swimming to sessile lifestyle transitions. Often, these transitions are governed by relative changes in intracellular cyclic-di-GMP concentrations. The VF_A0360 mutant exhibited a five-fold increase in intracellular cyclic-di-GMP concentrations compared to the parent strain, providing evidence that VF_A0360 regulates free-swimming to sessile lifestyle transitions in the host environment through modulation of intracellular cyclic-di-GMP.Current studies are aimed at (1) identifying genes regulated by VF_A0360 with RNA-seq; and (2) determining components involved in the downstream signaling pathway necessary for cyclic-di-GMP regulation.

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Identification of a host-specific locus found in the digestive tract microbiota of medicinal leeches

SOPHIE M. COLSTON[1], LINDSEY BOMAR[2], JOERG GRAF[1]

[1] *University of Connecticut, Storrs, CT*
[2] *The Forsyth Institute, Cambridge, MA*

The microbial community found within the gut of the medicinal leech, *Hirduo verbana*, is relatively simple and dominated by two bacteria, *Aeromonas veronii* and *Mucinivorans hirudinis*. *A. veronii* leech isolates appear to be highly adapted to a lifestyle within the leech crop since isolates from other sources exhibit a diminished capability to colonize the leech. These observations led to the hypothesis that there are discrete genetic loci that facilitate the successful proliferation and persistence of leech-associated *Aeromonas* within its host. We sequenced the genomes of 80 strains, primarily *A. veronii* strains from various sources and other leech-associated isolates identified as *A. jandaei, A. hydrophila*, and species from the genera *Proteus* and *Morganella*. Analysis of the genomic data revealed that the genes, nanAREKT, involved in the catabolism of sialic acid (SA), is predominantly exclusive to leech isolates. Gene expression values from a study to investigate host-microbe physiologies indicated that the nan catabolism genes are highly expressed at 8 h after feeding, denoting a nutritional role for SA. In vitro growth experiments demonstrated the ability of *A. veronii* leech isolates to utilize SA as a sole nutrient source. Mutants in genes required for SA catabolism were either unable or slow to grow on SA alone *in vitro*. These mutants did not have colonization defects in the leech under our assay conditions, which indicates that they are employing alternative nutrient utilization pathways. Many *Aeromonas* leech isolates also encode for a putative sialidase, an enzyme that cleaves SAs from their glycoconjugates, and is a unique feature in sialic acid specificity by serving as a scavenger for bound SAs. While SA catabolism may be a singular capability adapted for life within the leech gut, for *A. veronii*, it is also likely a metabolic redundancy that allows the bacterium to outcompete other strains within that environment.

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NPC proteins are key players in molecular interactions between cnidarians and their dinoflagellate endosymbionts

VINCENT DANI[1], FABRICE PRIOUZEAU[1], SOPHIE PAGNOTTA[2], SANDRA LACAZE-GERVAIS[2], JEAN-PIERRE LAUGIER[2], MARJOLIJN MERTZ[3], CÉCILE SABOURAULT[1]

[1] *Université de Nice-Sophia-Antipolis, UMR7138 Evolution Paris-Seine, Valrose, BP71, 06108 Nice Cedex 02, France*
[2] *Centre Commun de Microscopie Appliquée, Université de Nice-Sophia-Antipolis, Nice, France*
[3] *Plateforme d'imagerie PRISM, Institu*

Trophic endosymbioses between cnidarians and photosynthetic dinoflagellates form the key foundation of reef ecosystems. Cnidarians host their photosynthetic dinoflagellate symbionts in gastrodermal cells, in a phagosome-derived vacuole. The establishment and maintenance of the symbiotic interaction is dependent on intimate molecular communications, including recognition and tolerance of symbionts, as well as adaptations for mutual transport and exchange of nutritional resources. However, environmental perturbations, such as an increase in seawater temperature, may disrupt this partnership, leading to the severe worldwide decline of coral reefs. Here, we will especially focus on the sterol-binding proteins Niemann-Pick C (NPC1 and NPC2), which have been identified by transcriptomic analyses in symbiotic sea anemones. While only one NPC2 gene is present in many metazoans, this gene has been duplicated in cnidarians (at least four copies of NPC2 are detected). However, only one gene (AvNPC2-d) was upregulated in symbiotic relative to aposymbiotic sea anemones *Anemonia viridis* and displayed higher expression in the gastrodermis (symbiont-containing tissue) than in the epidermis. This gene was also strongly down-regulated in response to stresses that further led to the symbiosis collapse. Moreover, proteomic analyses of symbiosome membranes demonstrated that both AvNPC1 and AvNPC2-d are localized at the symbiotic interface. Several studies demonstrated in other biological models the involvement of NPC proteins in i) innate immune pathways, such as the filovirus entry in mammalian cells or the binding of bacterial cell wall molecules in *drosophila*, and ii) chemical communication, such as the binding of semiochemicals in worker ants. In symbiotic cnidarians, NPC proteins may be involved in sterol trafficking but may also participate in symbiont selection and/or symbiont persistence, for example by recognizing dinoflagellates cell-surface glycans. This may explain why several NPC2 genes have been retained in cnidarian genomes. This work was supported by the French ANR grant ANR-12-JSV7-0009-01 (inSIDE project)

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Symbiont diversity and its influence on host metabolite profile in a model cnidarian-dinoflagellate symbiosis

JENNIFER L. MATTHEWS[1], CLINTON A. OAKLEY[1], ARTHUR R. GROSSMAN[2], VIRGINIA M. WEIS[3], SILAS VILLAS-BOAS[4], SIMON K. DAVY[1]

[1] *Victoria University of Wellington, New Zealand*
[2] *Stanford University, USA*
[3] *Oregon State University, USA*
[4] *University of Auckland, New Zealand*

Metabolic exchange is central to the ecological success and stability of the cnidarian-dinoflagellate symbiosis and may also play a role in host-symbiont specificity. However little is currently known about this complex nutritional relationship. We infected the sea anemone *Aiptasia* sp. - a model system for reef-building corals - with homologous (naturally occurring) and heterologous (novel) symbiont types (*Symbiodinium* spp.). These types represented a diverse range of thermal tolerances, to shed some light on how thermally resistant versus susceptible symbionts influence the host's metabolite profile, and what this could mean for the potential survival of novel host-symbiont combinations that might arise after coral bleaching events. Metabolites were extracted from both host and symbiont fractions before separation into hydrophilic and lipophilic fractions. The metabolites were chemically derivatized and analyzed using Gas Chromatography separation linked to Mass Spectrometry (GC-MS). Qualitative and semi-quantitative multi-component analyses were used to describe metabolic patterns for metabolically- or analytically-related compounds; in this case photosynthetic products likely to be translocated from the dinoflagellate symbionts to the host. We observed clear differences in the metabolite profiles of the homologous and heterologous host-symbiont combinations, including a smaller diversity of fatty acids in the heterologous partnerships. This evidence indicates that symbiont type (including more thermally-resistant types) can negatively impact the host's metabolite profile in the cnidarian-dinoflagellate symbiosis, with potential consequences for the establishment and persistence of new mutualistic pairings in the field.

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Are beewolf symbionts fighting a losing battle against the radical host protection?

TOBIAS ENGL[1], TARAS NECHTAYLO[1], NATALIE WIELSCH[2], ERHARD STROHM[3], ALEŠ SVATOŠ[2], MARTIN KALTENPOTH[1]

[1] *Research Group Insect Symbiosis, Max Planck Institute for Chemical Ecology, Jena, Germany*
[2] *Research Group Mass-Spectrometry, Max Planck Institute for Chemical Ecology, Jena, Germany*
[3] *Institute for zoology, University of Regensburg, Germany*

One of the yet unanswered questions of symbiosis research is the evolutionary maintenance of symbiosis. Microbial symbionts suffer often not only from general degeneration effects of a symbiotic lifestyle like genome erosion and emergence of cheaters, but the host biology can hold its very own hazards. Beewolves engage since 68 million years in a defensive symbiosis with Candidatus *Streptomyces philanthi* (CaSP) to protect the late development of their offspring against mold fungi. In addition, the wasp egg initially releases high concentrations of the radical gas nitric oxide (NO) into the brood cell. NO forms highly reactive intermediates in the presence of oxygen radicals that also exert a potent antimicrobial effect and suppress especially fungal growth. However, the CaSP symbiont is also exposed to NO. We were thus interested, how it deals with the repeated NO stress physiologically as well as evolutionarily as also it undergoes several bottlenecks in each host life cycle with divergent selection pressures. Its metabolic versatility is already vastly limited by a dramatic accumulation of genomic defects. However, a high number of enzymes involved in nitrosative and oxidative stress response, including radical scavengers, superoxide/peroxide and NO detoxifying enzymes, high levels of general and stress induced chaperones as well as proteins binding and thereby protecting DNA and RNA/ribosome retain their functionality and are expressed on RNA and protein level both *in vitro* and situ. Thus, for the moment, the beewolf symbionts seem to be well set against their host's harsh treatment, although, the genome erosion including a severely impaired DNA repair machinery suggest an evolutionarily equivocal, though scientifically interesting perspective.

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Possible role of C-Type lectins in the establishment of Cnidarian-Dinoflagellate symbiosis

LISL Y. ESHERICK[1], ERIK LEHNERT[1], ELIZABETH HAMBLETON[2], SEBASTIAN BAUMGARTEN[3], AND JOHN R. PRINGLE[1]

[1] *Department of Genetics, Stanford University School of Medicine*
[2] *Centre for Organismal Studies, Heidelberg University*
[3] *Red Sea Research Center, King Abdullah University of Science and Technology*

It is clear that the cnidarian-dinoflagellate symbiosis is highly specific: a given host can establish a stable symbiosis with only some types in the diverse *Symbiodinium* genus. However, the cellular and molecular mechanisms underlying this specificity are poorly understood and must be clarified in order to understand the establishment and possible flexibility of this ecologically critical symbiosis. During the initial establishment of symbiosis in larvae, or during its reestablishment in adult animals that have lost their symbionts, the hosts must recognize compatible dinoflagellates among a diversity of microbes in the environment. Several studies have suggested the hypothesis that recognition depends on the binding of extracellular host lectins to oligosaccharide glycans on the dinoflagellate cell surface. Using the small symbiotic sea anemone *Aiptasia* as a model system, we have identified by transcriptome sequencing and qRT-PCR several genes encoding lectin-like proteins that are differentially expressed between symbiotic and aposymbiotic (without dinoflagellates) anemones. Notably, a majority of these genes showed increased expression in aposymbiotic anemones, suggesting that hosts may up-regulate genes involved in pattern-recognition when lacking symbionts. One such gene, encoding the C-type-lectin-like protein Ctl1, is expressed eight-fold higher in aposymbiotic animals. We have expressed a recombinant GST-tagged form of Ctl1 in bacteria and found that it binds strongly to an incompatible strain of *Symbiodinium in vitro*, though it also binds weakly to a compatible strain. This differential binding suggests that Ctl1 may play a role the host's ability to discriminate between compatible and incompatible symbiont types. Analysis of the *Aiptasia* genome has revealed that Ctl1 is a member of a family of related lectins originating from at least six genes in a tandem array. The binding properties of these related lectins are still unknown. Genetic methods will almost certainly be required to unravel the complexities of this system.

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Dual function of antimicrobial peptides in insect endosymbiosis: Does the bacteriome immunity have a ‘split personality’?

FLORENT MASSON[1], AGNÈS VALLIER[1], SÉVERINE BALMAND[1], CAROLE VINCENT-MONÉGAT[1], AURÉLIEN VIGNERON[1], ANNA ZAIDMAN-RÉMY[1] AND ABDELAZIZ HEDDI[1]

[1] *Laboratoire Biologie Fonctionnelle, Insectes et Interactions, INSA de Lyon*

Insects thriving on nutritionally unbalanced habitats often harbor mutualistic intracellular bacteria (endosymbionts) that complement their diet and improve their ecological performances. Specialized host cells, called the bacteriocytes, house endosymbionts and isolate them from the insect systemic immune response. In *Sitophilus* weevils, bacteriocytes group together to form the bacteriome organ, which expresses an immune program allowing endosymbiont tolerance and control. In standard developmental conditions, the bacteriome generates a modulated immune response directed towards endosymbionts. This response leads specifically to the expression of Coleoptericin A, an antimicrobial gene that secludes the endosymbionts inside the bacteriocytes. We recently determined that several immune effector genes, including other antimicrobial peptide coding genes, are activated in the bacteriome when insects are challenged with bacterial pathogens. Although this bacteriome immune response to external pathogens involves the same genes that the gut local immune response, the expression of effector genes is quantitatively lower in the symbiotic organ. The endosymbiont load is affected neither by the infection itself nor by the activation of effector genes, indicating that the constitutive symbiont-controlling process is not disrupted by the induction of the IMD-like defensive response.We speculate that the contained immune effector gene expression in the bacteriome may prevent potentially harmful effects of the immune response on endosymbionts, whilst efficiently protecting them and their host cells from pathogen intruders. Strikingly, our latest results show that the symbiont-controlling immune response is also mediated by an IMD-like pathway, involving the same transcription factor as the defense response. This points to a dual function of the IMD-like immunity in the bacteriome: controlling the load and localization of endosymbionts whilst protecting them from exogenous pathogen intruders. We currently are investigating the switch mechanism that allows these distinct functions to be assured by what appears at first sight to be the same signaling pathway.

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Of chance and necessity: A new suite of fungal-algal mutualisms created via synthetic ecology

ERIK F. Y. HOM

University of Mississippi

Mutualistic symbioses are widespread and shape the evolution of genomes and ecosystems. Despite their ubiquity and importance, we understand surprisingly little about how such symbioses arise. In part, the dearth of experimentally tractable systems has made it difficult to address fundamental questions about how symbiotic relationships arise, evolve, and are consolidated. Inspired by lichens, symbiotic composites involving fungi and algae, I devised a suite of obligate mutualisms based on a simple carbon:nitrogen metabolic exchange, with the prototype being between the model eukaryotes, *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii*. The capacity for mutualism is robust to variations in carbon and nitrogen nutrient concentrations, is phylogenetically broad among free-living fungi and green algae, and can serve as a basis for creating synthetic symbiotic "tissues" whereby algae physically associate with filamentous fungi. I will present the logic of my approach and describe how this set of mutualisms set the stage for further molecular, genetic, and experimental evolution studies on the origins and progression of nascent symbioses.

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On the origin and evolution of a tripartite nested mealybug symbiosis

FILIP HUSNIK[1,2], JOHN P MCCUTCHEON[1,3]

[1] *University of Montana, Division of Biological Sciences, Missoula, USA*
[2] *University of South Bohemia, Faculty of Science, Ceske Budejovice, Czech Republic*
[3] *Canadian Institute for Advanced Research, Toronto, Canada*

Mealybugs are sap-sucking hemipteran insects which depend on bacterial endosymbionts to provide them with essential amino acids missing from their phloem diet. One subfamily of mealybugs (*Pseudococcinae*) harbors a unique symbiotic system of one endosymbiotic betaproteobacterium (*Tremblaya princeps*) containing its own gammaproteobacterial endosymbionts (called *Moranella endobia* in *Planococcus citri*). This symbiosis is highly metabolically interconnected and supported by both symbiont and host enzymes—including genes horizontally transferred from other bacteria to the host genome. *Tremblaya princeps* from *Planococcus citri* mealybug also posseses a tiny 139 kb genome with the lowest number of genes reported from an insect endosymbiont or any other bacterium not called an organelle. Here, using host-symbiont genome data from six mealybug species covering all main clades of *Pseudococcinae* phylogeny, we interrogate fundamental questions about the origin and evolution of this unique nested symbiosis. When in the mealybug evolution was the first such tripartite arrangement established? Why was the intrabacterial symbiosis started? And, perhaps most importantly, how has it been evolving and maintained since then?

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Symbiont mechanisms for stabilizing an insect-microbe symbiosis with horizontal transmission

YOSHITOMO KIKUCHI, IKUMI NISHI, AND HIDEOMI ITOH

AIST, Japan

A number of animals and plants maintain specific symbiotic associations with benevolent microorganisms, even though the symbionts are not vertically transmitted (i.e. transmission from mother to offspring) but acquired from the environment every generation. In such associations, it has been reported that host organisms develop elaborate “partner-choice” mechanisms to winnow favorable symbionts from environmental microbiota, and “partner-fidelity” or policing mechanisms to stabilize the mutualistic cooperation. However, in contrast to the growing body of knowledge about host mechanisms, symbiont adaptations for maintaining the intimate associations remain unclear. On the basis of experimental inoculation of symbiotic *Burkholderia* and allied non-symbiotic bacteria into the bean bug *Riptortus pedestris*, we demonstrate here that colonization competitiveness is a pivotal symbiont mechanism for stabilizing the insect-microbe symbiosis. *R. pedestris* exclusively acquires *Burkholderia* symbionts from environmental soils and houses them in midgut crypts. The genus *Burkholderia* is among the most diverse groups in the *Proteobacteria*, wherein the insect symbionts form a monophyletic clade. Experimental inoculation of diverse bacterial species including *Actinobacteria*, *Firmicutes* and *Proteobacteria* in addition to diverse *Burkholderia* species, including symbiotic and non-symbiotic species, revealed that members of the genera *Burkholderia* and allied *Pandorea* could colonize the midgut crypts. However, while the natural symbionts filled the lumen of the crypts entirely, the non-symbiotic species only partially colonized the symbiotic organ. Surprisingly, despite the abnormal colonization phenotype, these non-symbiotic species promoted host growth and survival as efficiently as the *Burkholderia* symbionts. Competitive inoculation assays, however, showed that the *Burkholderia* symbionts consistently overwhelmed the non-symbiotic species in vivo. These results demonstrate that competitive ability, but not infectious ability and host fitness effects, is a characteristic trait of the symbiont lineage, which may make the *Riptortus*-*Burkholderia* symbiosis robust and stabilize the association.

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Sphingolipids in cnidarian-dinoflagellate interactions: investigating the role of the sphingosine rheostat during symbiont colonization

SHEILA KITCHEN, ANGELA POOLE AND VIRGINIA WEIS

Oregon State University

Lipids play a central in symbiosis, providing both cellular structure and energy storage, but little is known about how signaling lipids participate in onset and establishment of symbiosis between cnidarians and dinoflagellates of the genus *Symbiodinium*. Signaling lipids, sphingosine and sphingosine-1-phosphate (S1P), play a pivotal role in determining cell fate, where increased sphingosine drives apoptotic activity within the cell while S1P promotes cell survival. The balance of these lipids creates the 'sphingosine rheostat' and intracellular concentrations result from enzymatic reactions of sphingosine kinase and sphingosine-1-phosphatase (SPPase). The sphingosine rheostat was proposed to regulate cnidarian symbiosis following the discovery of differential expression of SPPase between symbiotic and aposymbiotic anemones. It was hypothesized that the dinoflagellate sends a tolerogenic signal to the host, which promotes cell survival through activation of sphingosine kinase, thus increasing cellular S1P. A recent study demonstrated that exogenously applied sphingolipids could alter this partnership, however endogenous regulation of the rheostat in cnidarians has not been characterized. Furthermore, sphingolipid metabolism has not been studied during onset of symbiosis. In this study, we investigated the role of sphingosine rheostat during symbiont colonization of the sea anemone, *Aiptasia*. The anemones were inoculated with *Symbiodinium* for one day and colonization dynamics monitored over three days. The expression of rheostat enzymes was examined using qRT-PCR. At early time points SPPase expression was up-regulated, which could correspond with initial symbiont contact and selection. Then, SPPase lowered expression later suggesting a shift toward cell survival. Sphingosine kinase expression did not significantly change throughout the experiment. To determine uptake of symbionts, we estimated relative algal densities using qRT-PCR. Finally, to link gene expression to enzymatic activity, we quantified sphingolipid concentrations using mass spectrometry analysis of lipid extracts. Collectively these data suggest that sphingolipid signaling plays a regulatory role in the onset of cnidarian-dinoflagellate symbiosis.

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Highly dynamic host regulation of *Symbiodinium* population during the ontogeny of the symbiotic scleractinian coral

A. LECOINTE[1,2], I. DOMART-COULON[2], A. MEIBOM[1,3]

[1] *Laboratory for Biological Geochemistry, EPFL, Lausanne, Switzerland.*
[2] *Molécules de Communication et Adaptation des Microorganismes, MNHN, Paris, France.*
[3] *Center for Advanced Surface Analysis, Institute of Earth Sciences, UNIL, Lausanne, Switzerland.*

Mutualistic endosymbiosis of shallow-water scleractinian corals with photosynthetic dinoflagellates is essential to the growth of most reef-builders, with several mechanisms involved in the steady-state maintenance of symbiont to host cell ratio. In the adult polyps, control of *Symbiodinium* sp. growth by the host includes nitrogen limitation by the host to prevent dinoflagellate cell division, and symbiont loss via induction of apoptosis, or direct detachment and expulsion. Early developmental stages, from planktonic planula larva to the benthic primary polyp and juvenile colony, provide the opportunity to investigate the ontogeny of the establishment and maintenance of endosymbiont cell homeostasis in coral tissue. Metamorphosis involves rapid tissue reorganization and growth, including a transition from two to four coral cell layers. The simultaneous control of intracellular *Symbiodinium* growth within this context has not been studied. Here we present an evaluation of cell proliferation and apoptosis, via BrdU pulse (24h) labeling and TUNEL immunodetection assays, in both coral and symbiont cells of *Stylophora pistillata* (Esper, 1797) during the different stages of metamorphosis. DNA synthesis activity in dinoflagellate cells is two to three times higher in planula and the primary polyp, compared with early metamorphosis or the adult stage. Their mitotic index peaks in the primary polyp. Dinoflagellate rate of apoptosis is relatively low in planula and early metamorphosis stages, at levels below or comparable to the adult stage, but peaks in the primary polyp. Overall, the turnover is higher for symbiont cells than for gastrodermal host cells, especially at the planula and primary polyp stages, but the relative abundance of dinoflagellates to coral gastrodermal cells remains stable at 6 ± 2% symbionts throughout metamorphosis. These data suggest highly dynamic host regulation of the symbiont population during the ontogeny of the symbiotic scleractinian coral, with increasing regulation through apoptosis.

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Endosymbiosis in trypanosomatid protozoa: The bacterium division is controlled during the host cell cycle

CAROLINA MOURA COSTA CATTA-PRETA[1], FELIPE LOPES BRUM[1], CAMILA CRISTINA DA SILVA[1], ALINE ARAUJO ZUMA[1], MARIA CAROLINA ELIAS[2], WANDERLEY DE SOUZA[1], SERGIO SCHENKMAN[3], MARIA CRISTINA M. MOTTA[1]

[1] *Instituto de Biofísica Carlos Chagas Filho, UFRJ*
[2] *Center of Toxins, Immunology and Cell Signaling, Instituto Butantan*
[3] *Escola Paulista de Medicina, Universidade Federal de São Paulo*

Co-evolution between primitive organisms associated by symbiosis offers valuable information about the eukaryotic cell evolution. Some trypanosomatids maintain a mutualistic relationship with a bacterium that divides in synchrony with other host cell structures. Genomic data showed that the symbiont suffered a massive gene loss, including those related to division and cell wall assembly. In this work, we study the coordinated division between the symbiont and the host protozoan by investigating the cell cycle in two trypanosomatids, which belong to divergent genera, *Angomonas deanei* and *Strigomonas culicis*. Protozoa were treated with cicloheximide that inhibits protein synthesis in eukaryotes and with different inhibitors that block cell cycle in distinct phases. Then, in order to establish cellular patterns and to verify cell cycle arrest, protozoa were submitted to immunofluorescence and electron microscopy techniques, as well as to cytometry flow analyses. Our results showed that cicloheximide treatment completely blocked trypanosomatid proliferation, but did not impeach symbiont DNA duplication. However, most bacterium maintained the constricted format, indicating that proteins produced by the host cell are required for the prokaryote division. Treatment with aphidicolin promoted protozoa cell cycle arrest in G1/S phase and the bacterium present in each species responded differently: in *A. deanei*, the symbiont duplicated its DNA and presented up to 4 nucleoids, whereas in *S. culicis* the bacterium acquired a filamentous structure with multiple nucleoids. Camptothecin treatment promoted protozoa cell cycle arrest in G2-phase and generated filamentous symbionts in both species. Oryzalin, which blocks microtubule polymerization, induced cell cycle impairment in mitosis and prevented symbiont division. Taking together, our results indicate that each host trypanosomatid control the bacterium division differently. In *A. deanei* the cell cycle coordination is established in G1/S phase, whereas in *S. culicis* it is induced later on, during mitosis.

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Metabolomic of mutualism establishment in planktonic photosymbiosis

LOIC PILLET[1], ANDRES GUTIERREZ[1], YANN GUITTON[2], MOHAMED MEHIRI[3], ESTELLE BIGEARD[1], TRISTAN BIARD[1], KARINE CAHIER[4], FANNY GAILLARD[4], DAMIEN EVEILLARD[2], FABRICE NOT[1]

[1] *UMR7144, CNRS-UPMC Station Biologique de Roscoff, Roscoff, France*
[2] *UMR 6241, University of Nantes, Nantes, France*
[3] *UMR7272, Institut de chimie de Nice, Nice, France*
[4] *FR2424, CNRS-UPMC Station Biologique of Roscoff, Roscoff, France*

Establishment of specific symbiotic associations between two organisms is an extremely complex process, involving a number of successive steps. This is particularly true in the case of intracellular symbioses between two unicellular eukaryotes, which represent the ultimate level of intimacy between a symbiont and its host. Among the most fundamental, and yet open question on this topic, is the ability of both partners to recognize specifically each other to initiate such interaction and avoiding the symbiont to be digested like a regular prey.In the present study, we investigated the molecular mechanisms underlying the establishment of symbiosis between colonial *Radiolaria* (*Collodaria*) and their specific algal symbionts, dinoflagellates from the genus *Brandtodinium*. Freshly collected radiolarian individuals were incubated in the presence or absence of different algal cultures, including *Brandtodinium* and other dinoflagellate species from genus *Pelagodinium*, which are not specific to radiolarian hosts. Samples from (1) the host, (2) the algal partner and (3) the incubating filtered seawater were collected at two different times of incubation. Endometabolomes of each symbiotic partner, as well as their exometabolomes contained in the incubating seawater were analyzed using mass spectroscopy techniques. In parallel, transcriptomic data are being acquired for both, the radiolarian host and the algal symbiont, in order to model the molecular pathways involved in mutual recognition.Comparative analysis of endo- and exometabolomes of the different partners in different experimental conditions overtime show differential patterns and preliminary results will be presented and discussed.

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Hemocyte response in the symbiosis between the Hawaiian Bobtail Squid, *Euprymna scolopes*, and the bioluminescent bacterium, *Vibrio fischeri*

TYLER SCHLEICHER, SARAH MC ANULTY, SPENCER V. NYHOLM

Department of Molecular and Cell Biology, University of Connecticut

The establishment and maintenance of the light organ symbiosis between the Hawaiian bobtail squid, *Euprymna scolopes*, and the bioluminescent bacterium, *Vibrio fischeri*, depends on selection of *V. fischeri* and exclusion of non-symbiotic bacteria from the environment. Current evidence suggests that the host's cellular innate immune system, in the form of macrophage-like hemocytes, assists in mediating symbiont specificity. In vitro assays that compared binding and phagocytosis of *V. fischeri* and non-symbiotic bacteria by juvenile and adult hemocytes suggest that these immune cells undergo a maturation process leading to specific recognition and tolerance of the symbiont. To further understand the role of hemocytes in this association, two independent quantitative proteomics techniques (isobaric tags for relative and absolute quantificaction (iTRAQ) and spectral counting) were used to analyze hemocytes from symbiotic and antibiotic cured hosts. Thirty-seven unique proteins were determined to be significantly different between sym and cured hemocytes (p value < 0.05), with 20 more abundant proteins and 17 less abundant in sym hemocytes. Comparisons between iTRAQ and spectral counting revealed that 30 of the 37 proteins quantified via iTRAQ exhibited trends similar to those identified by the label-free method. Colonization state influenced the abundance of proteins associated with the cytoskeleton, adhesion, lysosomes, proteolysis, and the innate immune response. One of these proteins is a novel peptidoglycan recognition protein (EsPGRP5) with conserved residues for predicted amidase activity. A recombinant EsPGRP5 protein degraded peptidoglycan from *V. harveyi* but showed less activity against *V. fischeri* and two Gram-positive strains tested, suggesting that EsPGRP5 may have variable activity towards different bacteria. Immunocytochemistry also revealed that EsPGRP 5 co-localizes with lysozomes in hemocytes. Together, these data suggest that light organ colonization influences production of host proteins that may be involved with mediating specificity in the association.

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The effects of symbiotic state on the proteome of the model cnidarian *Aiptasia pulchella*

CLINTON A. OAKLEY[1], MICHAEL AMEISMEIER[2], LIFENG PENG[1], VIRGINIA M. WEIS[3], ARTHUR R. GROSSMAN[4], AND SIMON K. DAVY[1]

[1] *School of Biological Sciences, Victoria University of Wellington.*
[2] *Gene Center and Dept of Biochemistry, Ludwig-Maximilians-Universität München.*
[3] *Dept. of Integrative Biology, Oregon State University.*
[4] *Dept. of Plant Biology, The Carnegie Institution.*

Coral reef ecosystems are metabolically based on the mutualism between corals and photosynthetic dinoflagellates, genus *Symbiodinium*. The glass anemone *Aiptasia pulchella* is a tractable model for this symbiosis which can be rendered free of symbionts and remain aposymbiotic in the laboratory. Label-free mass spectrometry-based "shot-gun" proteomics is a powerful technique for the simultaneous identification and quantification of large numbers of proteins, providing data that are more directly related to physiological function than transcriptional approaches. We utilised label-free liquid chromatography-tandem mass spectrometry (LC MS/MS) to analyse the effects of symbiosis on the proteomes of symbiotic and aposymbiotic *Aiptasia*. We both identified and achieved relative quantification of over 1,200 proteins from the host. Symbiotic anemones showed a significantly higher expression of lipid storage and transport proteins, nitrogen transport proteins, carbonic anhydrase, and central metabolism enzymes. These changes likely reflect shifts in host metabolism and reserves due to the increased energy supplied by the symbionts, as well as mechanisms to supply inorganic nutrients to the symbionts. In contrast, aposymbiotic anemones had an increased expression of an array of proteases, including chitinase, which may indicate a potential heterotrophic "priming" response to lower nutrient availability. These results corroborate previous studies as well as providing a more accurate relative quantification of protein abundance. The expansion of modern proteomics techniques into this model system will allow more powerful studies into coral physiology and the effects of biotic and abiotic stress on the coral/dinoflagellate mutualism.

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Session: Establishment and maintenance of mutualism
Presentation type: Oral
Name, Contact: Ohbayashi Tsubasa, tsubasa-oobayashi@aist.go.jp

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The role of crypt-specific cysteine-rich proteins (CCRs) in the bean bug-*Burkholderia* symbiosis

TSUBASA OHBAYASHI[1], PETER MERGAERT[1], [2], RYO FUTAHASHI[3], YU MATSUURA[1,3], MIA TERASHIMA[1], YOICHI KAMAGATA[1], [3], TAKEMA FUKATSU[3], YOSHITOMO KIKUCHI[3]

[1] *Hokkaido University, JAPAN*
[2] *CNRS, France*
[3] *AIST, Japan*

Many insects possess symbiotic bacteria within specific organs. These symbionts are known to be important for host survival, whereas molecular mechanisms underpinning symbiotic interactions remain poorly understood, because many insect-microbe symbioses are not easily accessible for functional analyses. The bean bug *Riptortus pedestris* develops symbiotic crypts in a posterior region of the midgut, where specific bacterial symbionts of the genus *Burkholderia* populate densely. Unlike previously well-studied insect symbionts, the *Burkholderia* symbiont is easily culturable and genetically manipulatable. Also the insect is amenable to reverse genetic techniques like RNAi. These features provide an unprecedented opportunity to elucidate the molecular basis of insect-microbe symbioses. We performed transcriptomic analyses of the midgut of symbiotic and aposymbiotic insects, thereby uncovering many genes specifically expressed in the crypts of symbiotic insects. Among them, a major gene family was identified encoding small secretion proteins named “crypt-specific cysteine-rich” proteins (CCRs). CCRs are characterized by specific patterns of six or more cysteine residues in their C-terminus. These CCRs displayed no homology to known proteins, but similar cysteine-rich patterns are found in antimicrobial peptides like defensins. Similar proteins, known as “nodule-specific cysteine-rich” proteins (NCRs), are also specifically expressed in root nodules, symbiotic organs in the legume-*Rhizobium* symbiosis. NCRs inhibit cell division of symbiotic bacteria, inducing their differentiation into a specialized nitrogen-fixing form called “bacteroid”. We observed that symbiotic *Burkholderia* cells were markedly smaller in size and DNA content than cultured *Burkholderia* cells, suggesting the possibility of host’s control over symbiont replication. We are currently investigating the expression of cell cycle genes in symbiotic and cultured *Burkholderia* cells, RNAi suppression of the CCR genes and its phenotypic consequences, and *in vitro* exposure of cultured *Burkholderia* cells to synthetic CCRs. On the basis of these results, we propose hypotheses as to what biological roles the CCRs play in the *Riptortus*-*Burkholderia* symbiosis.

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General models of the evolution of interspecific interactions

DAVID C. QUELLER

Department of Biology, Washington University in St. Louis

One important role of evolutionary models, besides providing detailed predictions, is to formalize major concepts in a simple and logically transparent manner. Examples include Fisher's fundamental theorem of natural selection for the process of adaptation, and Hamilton's rule for social interactions among conspecifics. Less attention has been given to evolutionary interactions between species. Here I develop a style of modeling for between-species interactions, focusing particularly on mutualisms, with the goal of reaching simple and general conclusions. I take two approaches, both starting with the Price equation. First, considering selection on a single species at time, I show how it can be partitioned, much like kin selection, into effects on own fitness and effects of partners, the latter multiplied by a structural feedback coefficient somewhat analogous to relatedness. Second, I consider simultaneous selection on two partners who affect a trait jointly. This approach yields results defining evolutionary conflict and cooperation, as well versions of Fisher's fundamental theorem for multi-species interactions.

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Symbiosome membranes characterization, or how to decipher molecular interactions between cnidarians and their dinoflagellate symbionts

CECILE SABOURAULT[1], VINCENT DANI[1], FABRICE PRIOUZEAU[1], JOHANA REVEL[1], MOHAMED MEHIRI[2], LIONEL MASSI[2], LAURE CAPRON[2], S PAGNOTTA[3], S LACAS-GERVAIS[3], J-P LAUGIER[3], K LEBRIGAND[4], P BARBRY[4], M WISZTORSKI[5], D MOUAJJAH[5], MICHEL SALZET[5]

[1] *Université de Nice-Sophia-Antipolis, UMR7138 EPS, 06108 Nice, France*
[2] *UNS, UMR7272, ICN, 06108 Nice*
[3] *UNS CCMA, 06108 Nice*
[4] *IPMC, UMR7275, 06560 Sophia Antipolis, France*
[5] *Université Lille 1, PRISM INSERM U1192, 59655 Villeneuve d'Asc*

Cnidarians host their photosynthetic dinoflagellate symbionts in the gastrodermal tissue layer, in a phagosome-derived vacuole called the symbiosome. Symbionts are therefore separated from the host cytoplasm by one outer symbiosome membrane of animal origin, plus a number of inner membranes of algal origin. Despite its critical role in the symbiotic interaction, little information is available on that symbiosome membrane complex. We report here the results from a multidisciplinary approach to characterize the symbiosome membrane complex in the sea anemone *Anemonia viridis*. Transmission electron microscopy, confocal imaging, proteomics, metabolomics and mass spectrometry imaging were combined to investigate this symbiotic interface. For example, we determined the identity of symbiosome membrane proteins using shotgun proteomics. These data provided new insights about the biological role of the symbiosome membrane proteins. Taken together, all these results will allow us to better understand the biochemical composition of the symbiosome membrane and thus provide a basis to hypothesize mechanisms of the symbiotic interaction.This work was supported by the French ANR grant ANR-12-JSV7-0009-01 (inSIDE project)

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Genomic complexities in evolution of the beneficial apicomplexan *Nephromyces* from parasitic ancestors

SERGIO A MUNOZ-GOMEZ[1], CHRIS PAIGHT[2], MARY BETH SAFFO[2], CHRISTOPHER LANE[2]; CLAUDIO H SLAMOVITS[1]

[1] *Dalhousie University*
[2] *University of Rhode Island*

As the only beneficial symbiont among the otherwise parasitic Apicomplexa, *Nephromyces*, a horizontally-transmitted symbiont in molgulid ascidian tunicates, presents an exceptional opportunity to illuminate factors correlated with evolution of mutualistic outcomes in animal-microbial interactions. To probe the evolution of this mutualism, we have begun a comprehensive genomic analysis of *Nephromyces*, with an initial aim of clarifying the phylogenetic relationships between *Nephromyces* and its parasitic relatives. We have found apicoplast genomes in *Nephromyces*, adding robust support to earlier data demonstrating its apicomplexan affinities. Apicoplast sequence data also suggest that *Nephromyces* is a divergent apicomplexan clade distinct from, and possibly basal to, “core” apicomplexan clades. 18S rDNA sampling thus far indicates that *Cardiosporidium*, a parasite found across a broad taxonomic range of non-molgulid ascidians, is a monophyletic clade sister to *Nephromyces*. The association of both clades with ascidian hosts, the presence of symbiotic bacteria (rare among apicomplexa) in both taxa, and the highly derived features of both *Nephromyces* and its molgulid hosts raise the possibility that the mutualistic *Nephromyces*-molgulid symbiosis evolved from an ancestral apicomplexan-ascidian parasitism. Apicoplast and 18S rDNA data reveal surprisingly high genomic diversity in *Nephromyces*, even in samples from a single host individual. Several biological features support the plausibility of multiple *Nephromyces* variants within single hosts. (a) Multiple infective stages of *Nephromyces* in *Molgula* blood after inoculation of lab-raised, symbiont-free animals and (b) the competence of *Molgula* for infection throughout post-metamorphic life suggest the likelihood of multiple *Nephromyces* infections in individual hosts; further (c), experimental cross-inoculations of symbiont-free animals with *Nephromyces* from three different *Molgula* species indicate that some *Nephromyces* strains may infect more than one *Molgula* species. The observed genomic complexity also suggests the possibility of functional diversity among different *Nephromyces* variants, and of complex evolutionary and metabolic dynamics of both symbiont-symbiont and symbiont-host interactions in this system.

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Stability and instability of host-symbiont combinations among higher fungus-gardening ants

JON SEAL, ULRICH G. MUELLER

Integrative Biology, University of Texas at Austin 1 University Station #C0930 Austin, TX 78712, USA

Background: A key feature of symbioses is stability and persistence across ecological and evolutionary scales. Although the so-called ‘higher-attini’ (the leaf-cutter ants (*Atta* and *Acromyrmex* spp.) and non-leaf-cutters (*Trachymyrmex* spp.) were widely considered to be a classic case of 1:1 coevolution because each of these two groups of ants grow distinct clades of derived ant fungi, large scale geographical surveys have shown that there are naturally occurring symbiont switches between these two ant and fungal clades. The mechanisms that promote or maintain these associations are currently unknown. Results: We report here the results of experimental symbiont switching (cross-fostering) from six North American fungus gardening-ant species. The six species show three generalized phenotypic responses when growing novel fungi. Some fail immediately, some sustain growth for several weeks before undergoing irrevocable declines, while others remain stable for 4 years or more. Because the unstable combinations become invaded by weedy, pathogenic species, we suspect that ant and fungal microbiomes may have a role in stabilizing ant and fungal combinations over ecological and evolutionary time scales. Significance: Fungus-gardening ants are excellent models to investigate the mechanisms involved in promoting symbiotic stability, because the main partners (ants and fungi) are macroscopic and can thus be disassembled and reassembled with relative ease. These symbioses are more tractable than other vertically transmitted symbioses where symbionts are often microscopic and reside inside their hosts. These studies are among the first experiments that examine the stability of host-symbiont pairings in fungus-gardening ants, especially those that involve interspecific switches where ants are paired with a distantly related fungus.

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Ancestral plant forms conquering terrestrial habitats: Survival, biodiversity, evolution of symbiotic lichenized and freeliving *Trentepohliales*

STOCKER-WÖRGÖTTER, E.

University of Graz, Institute of Plant Sciences, Holteigasse 68010 Graz, Austria

Most representatives of green algae are aquatic organisms, found in marine or freshwater environments. As the majority of the *Trentepohliales* is adapted to increased temperatures and high moisture contents, they are especially important and likely diverse, free-living and symbiotic as photobionts, in lichens of the tropics. A smaller number of *Trentepohliales* evolved strategies to survive at cooler temperatures and in temporarily dry habitats. During evolution such and other adaptations may have been important for a transition from life in water to life on land; *Trentepohlia* algae could be interpreted as transitional stages in the shift from aquatic organisms to land plants, long before the first vascular plants appeared. By colonizing terrestrial substrates, they can survive conditions with fluctuating levels of air humidity. The majority of *Trentepohliales* tested in our lab, can be cultured in liquid and solid nutrient media, as is shown in culture experiments, which have been performed together with phylogenetic relationship analyses to determine the identity of the different algal species. By colonizing various ecological niches in the free-living state, *Trentepohliales* have adapted to even more stressful conditions when living in a lichen thallus. In general, organisms, especially when adopting the symbiotic life style show enormous resistance and maintenance of mutualism, as twopartite or multipartite associations, able to survive extreme levels of abiotic stress. Not by surprise, *Trentepohliales* constitute the photobionts of approximately 50% of all lichenized fungi. The orange colour of the algal filaments, which masks the green of the chlorophyll, is caused by carotenoid pigments. Astaxanthin (former haematochrome) has been found to be one of “nature’s super carotenoids”. *Trentepohliales* comprise the widest spectrum of accumulated alditols reported for any algal group; thus providing attractive carbohydrate sources for their fungal partners. Morphological, molecular data sets, lifestyle analyses elucidating the origins of diverse Trentepohlialean symbioses will be presented.

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Name, Contact: Strassmann Joan, strassmann@wustl.edu

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Interactions between the social amoeba *Dictyostelium discoideum* and soil bacteria

JOAN E. STRASSMANN[1], DEBRA A. BROCK[1], USMAN BASHIR[1], FISHER BRODIE[2], JESSE GALLOWAY[2], & DAVID C. QUELLER[1]

[1] *Biology Department, Washington University in St. Louis, Missouri, USA*
[2] *Mountain Lake Biological Station, University of Virginia, Virginia, USA*

Ancient symbioses are responsible for some striking features of life, from coral reefs to tropical forests. But how symbiosis arises and how the interests of symbiotic partners are met, or defeated, is more easily studied in systems with recent or variable partnerships. This is particularly true for symbioses between eukaryote soil amoebae and the bacteria they encounter and sometimes harbor. After all, bacteria can become part of the amoebae that ingest them simply by defeating a few fragile membranes. Recently we discovered that some clones of the social amoeba *Dictyostelium discoideum* have a tripartite farming symbiosis with food bacteria and defensive bacteria (1,2). Intensive investigation into interactions with the food bacterium *Klebsiella pneumoniae* and naturally carried defensive bacteria close to *Burkholderia* xenovorans indicate behavioral shifts in amoebae with symbionts. Furthermore, interactions with other bacteria depend on relative densities of amoebae and bacteria. (3).Here we describe these interactions, then explore what bacteria are picked up by *D. discoideum* amoebae and carried through the social stage as temporary passengers or long-lasting symbionts. In over 40 samples of soil and deer scat from Mountain Lake Biological Station, we discovered over 200 bacterial isolates from *D. discoideum* fruiting bodies. Some can maintain amoeba growth, but some cannot; some are stable symbionts, but most are not. Investigations of these samples can tell us much about the origins of stable symbiosis.

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Growing faster or growing too fast? Effects of *Lactobacillus plantarum* on *Drosophila* fitness

MÉLISANDRE TÉFIT, FRANÇOIS LEULIER

Institut de Génomique Fonctionnelle de Lyon IGFL, Ecole Normale Supérieure de Lyon, CNRS UMR5242

Metazoans are naturally associated with bacterial communities, which establish commensalistic or mutualistic relationships with each other and with the host organism. These interactions play a crucial role in different aspects of the host physiology while providing the microbiota a nutrient-rich environment. When deregulated, the relationships can lead to pathological situations, such as inflammatory chronic disease, metabolic disorders or even cancers, and despite recent progress, the characteristics and molecular basis of the beneficial impact microbiota has on its host remain largely unknown.In order to elucidate the fine-tuned dialogue governing the relationships between intestinal bacteria and their host, we use the model system *Drosophila melanogaster* associated with one of its natural commensals, *Lactobacillus plantarum*. This rather simple gnotobiotic model allowed us to reveal a growth-promoting effect mediated by *L. plantarum* in nutritionally challenged *drosophila* larvae. In case of nutrient scarcity, larvae associated with *Lactobacillus plantarum* develop twice as fast as germ-free larvae, giving rise to adults of similar weight and size. But adjusting development to environmental cues is key to organismal fitness, and accelerated growth upon nutritional challenge might adversely impact adult fitness.In this light, we studied different life history traits and showed that adults emerging from fast growing larvae perform as well as their slow growing axenic siblings. They were able to resist starvation equally well and produced equally numerous and fit progeny. Here we show that *Lactobacillus plantarum* acts as a true mutualist of *Drosophila melanogaster*, as it allows the precocious emergence of a mature and fertile adult without fitness drawbacks.

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Short-term and long-term acclimation to ocean acidification of symbiotic cnidarian *Anemonia viridis*

PATRÍCIA VENTURA[1,2,3], MIKE JARROLD[4,5], PIERRE L. MERLE[1,2,3], STÉPHANIE BARNAY-VERDIER[1,2,3], THAMILLA ZAMOUM[1,2,3], RICCARDO RODOLFO-METALPA[6], PIERO CALOSI[4,5], PAOLA FURLA[1,2,3]

[1] *Université Nice Sophia Antipolis, UMR 7138 "Evolution Paris Seine", équipe "Symbiose marine", 06108 Nice Cedex 02, France,*
[2] *Université Pierre-et-Marie-Curie, UMR 7138 "Evolution Paris Seine", 7, quai Saint-Bernard, 75252 Paris cede*

Non-calcifying symbiotic cnidarians are a group of organisms than can thrive under ocean acidification (OA) conditions. However, it is unclear which physiological mechanisms drive this success in an elevated CO_2 environment. Symbiotic cnidarians express carbonic anhydrase enzymes, responsible for the reversible conversion of bicarbonate into carbon dioxide, which facilitates the initial DIC (dissolved inorganic carbon) absorption in the seawater and participates to the mechanisms of inorganic carbon concentration. Recent studies on non-calcifying symbiotic cnidarians demonstrated that elevated CO_2 conditions had no effect on photosynthetic activity. However, up to now is still unclear how OA conditions affect the autotrophic relationships in the symbiosis. In our study, we assessed the impact of increased pCO_2 in the activity and expression of animal carbonic anhydrase enzymes, in the symbiotic temperate sea anemone *Anemonia viridis*. We investigated *in situ* long-term exposure as a proxy to adaptation to OA conditions and in aquaria short-term exposure (3 weeks) as a proxy of acclimation. For both short and long-term exposures, animal CA activity was significantly lower at higher pCO_2 suggesting a change in physiological properties of the cnidarian cells. Our results suggest that non-calcifying photosynthetic cnidarians can adapt to long-term exposure to elevated CO_2 levels and that acclimation is possible even after 3 weeks. These data point out the great plasticity of this symbiotic phototrophic organism to OA conditions.

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Role of innate immunity in the regulation of cnidarian-dinoflagellate symbioses

VIRGINIA M. WEIS

Department of Integrative Biology, Oregon State University

Corals engage in a mutualistic symbiosis with intracellular photosynthetic dinoflagellates. This intimate partnership forms the trophic and structural foundation of coral reef ecosystems. This presentation will examine the cellular and molecular mechanisms underlying the establishment, maintenance and breakdown of the symbiosis in coral- and anemone-dinoflagellate partnerships. Host innate immunity and symbiont strategies for modulating this immune response are central to the stability of the symbiosis. During onset and maintenance of symbiosis these mechanisms include, lectin-glycan signaling, upregulation of the immunosuppressive TGFβ pathway and changes in the sphinolipid rheostat. Coral bleaching, a severe threat to the health of reefs worldwide, is caused by global warming and results from the dysfunction and collapse of the symbiosis. Several studies suggest that coral bleaching is a host innate immune response to a symbiont compromised by severe oxidative stress. This evidence includes increased nitric oxide levels, and host cell apoptosis and autophagy in heat-stressed animals, all well-known immune mechanisms in other systems to eliminate detrimental microbial invaders.

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The type 3 secretion system (T3SS) improves bacterial fitness in the mycosphere

PU YANG, MIAOZHI ZHANG[1], JAN DIRK VAN ELSAS

Department of Microbial Ecology, Groningen Institute for Evolutionary Life Science, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, the Netherlands

Background: The type three secretion system (T3SS) is a protein delivery system in bacterial pathogenesis. It can also mediate biofilm formation of bacterial cells on plant or animal surfaces. Recent studies suggested that T3SS might be involved in selection of bacterial populations by soil fungi. *Burkholderia terrae* BS001 is a T3SS-positive strain that is able to migrate along with growing fungal hyphae and form biofilms around the fungal hyphae. In this study, we made a T3SS knock-out strain of *B. terrae* BS001 to investigate if T3SS is involved in the interaction with fungi in mycosphere. Materials and methods: SctD, a key component of T3SS structural complex, was knocked out via double crossover allelic exchanges. The mutant strain was introduced into soil microcosms alone or together with the wild-type strain to investigate the migratory effect along with fungal hyphae. Results: 1 kb of sctD gene was deleted successfully. The mutant strain was still able to disperse through soil along with growing fungal hyphae of *Lyophyllum* sp. strain Karsten and *Trichoderma asperellum* 302. However, when wild-type and mutant strains were introduced into the soil microcosm together, the wild-type became much more abundant in the migration site compared to the inoculation site. These results demonstrated that fungal hyphae selectively carry more wild-type cells than mutant cells with them. Thus the wild-type strain won in the competition with mutant strain and dominated the new niche. Conclusion: The T3SS plays a beneficial role in the interaction of *B. terrae* BS001 with soil fungi and improves bacterial fitness in the mycosphere. However, T3SS is not the only mechanism involved in the interactions, the mechanisms exerted in the interaction were complex. Significance: As far as we know, this is the first time that the role of T3SS in the selection of bacteria by soil fungi is verified.

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Transmission of dominant *Wolbachia* and *Mollicutes* gut bacteria in leaf-cutter ants

MARIYA V. ZHUKOVA, PANAGIOTIS SAPOUNTZIS, MORTEN SCHIØTT, JACOBUS J. BOOMSMA

Centre for Social Evolution, Department of Biology, University of Copenhagen

Significance. We characterized the early establishment of gut bacterial communities in two leaf-cutter ant species, *Acromyrmex echinatior* and *Atta cephalotes*, both important herbivores in the New World tropics. Colonies of eusocial insects are second in complexity only to human societies and among these leaf-cutting ants stand out because they farm their own fungal food. Specialized gut bacterial communities have become established, but their function and transmission across life stages are largely unknown. We used 16S metagenome sequencing to determine the composition of gut bacterial symbionts in larvae and pupae. Our data show that *Wolbachia* OTU is the dominant species in *A. echinatior* at all developmental stages, while *A. cephalotes* lacks *Wolbachia* and has a higher gut bacterial diversity with abundant *Mollicutes* in adult workers. *Wolbachia* appears to be transovarially transmitted in *A. echinatior* as in most solitary insects, but *Mollicutes* are absent in eggs, present in guts of some larvae and disappear from the pupal gut during metamorphosis, while remaining present in other pupal tissues. This suggests that *Mollicutes* are transmitted to the guts of adult workers via indirect pathways after hatching: through haemolymph, fungus garden or by trophallaxis. Larval and pupal guts of both species also contain dominant bacterial species found in fungus gardens (*Pseudomonas* and *Enterobacter*) while adult workers do not or hardly have these bacteria. These fungus garden bacteria may thus be beneficial for larval growth and development, but not for the functional performance of adult workers, but resolving this will need further research.Conclusions. The bacterial gut communities of adult workers differs from the gut communities of larvae and pupae in both leaf-cutting ant species, suggesting host bacterial specificity that is changing during development and potentially different needs for mutualistic services across the life stages that are mediated by either vertical or social transmission of bacteria.

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Session Chairs: [1]James White, [2]Charles Bacon, [3]Russell J. Rodriguez
Chair affiliations: [1]Rutgers University, USA; [2]Russell Research Center, USA
[3]Adaptive Symbiotic Technologies, USA

Functional plant microbiomes: Effects of endophytes and epiphytes on plants

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Culturable endophytic bacteria of pea (*Pisum sativum* L.)

AKHTEMOVA GULNAR, AFONIN ALEKSEY, NOVOSELOVA NATALIA, ZHUKOV VLADIMIR, BORISOV ALEKSEY, TIKHONOVICH IGOR

Federal State Budget Scientific Institution All-Russia Research Institute for Agricultural Microbiology

Bacterial ability to use internal environment of plants (endosphere) as an ecological niche has led, in the process of evolution, to the formation of plant-microbe symbioses. Almost all plants contain endophytic bacteria, each separate species being a host to one or more endophytic organisms. These bacteria generally colonize plant intracellular spaces and can be isolated from all parts of a plant, including its seeds. Currently plant endomicrobiomes are practically not studied.Aim of this work: to isolate and study culturable bacterial endophytes of pea (*Pisum sativum* L.) for the development of microbial preparations. Endophytic bacteria were isolated from stems and seeds of pea (*Pisum sativum* L.) of the following genotypes: JI 2822, VIR 8274 and a commercial pea cultivar "Triumph" (VNIIZBK). Seeds and stems were sterilized in 70% ethanol and sodium hypochlorite and subsequently were placed on solid agar media TSA, 1/20 TSA, R2A. 135 different strains of endophytic bacteria were isolated: 62 from seeds of Triumph cultivar, 33 from seeds of JI2822 line, 30 from seeds and stems of VIR 8274 line.Agronomically-beneficial traits (plant growth promoting activity, production of auxin-like compounds, fungicidal and antibiotic activity) were found in 18 strains. Molecular identification of these strains was performed by sequencing of V1-V9 variable regions of 16S rDNA amplified with universal primers.Endophytic bacteria found in pea belong to 8 genera: *Bacillus* (majority of species), *Stenotrophomonas*, *Microbacterium*, *Paenibacillus*, *Kocuria*, *Staphylococcus*, *Micrococcus*, *Actinobacterium*. Endophytes belonging to the genus *Bacillus* found in all pea genotypes dominated respective endomicrobiomes, most agronomically-beneficial being bacteria isolated from pea stems.Thus, different tissues of pea contain culturable strains of bacterial endophytes possessing plant growth promoting properties that can be used for the production of microbial fertilizers.

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Endophytic microbiomes: Defensive functions and responsible metabolites of microbial endophytes

CHARLES W. BACON, DOROTHY M. HINTON, TREVOR R. MITCHELL

USDA, ARS, US National Poultry Research Center, Toxicology & Mycotoxin Research Unit

Increasing evidence indicates that plant microbiomes influence the ecological success of plant hosts. Further, endophytic microbes such as bacteria and fungi greatly affect plant stress tolerance and are responsible for defensive reaction to several forms of herbivory. What is not yet clear is whether it is an evolutionary strategy for plants to seek out and selectively obtain microbes for their ecological benefits. Several questions can be asked about plant microbiomes. Are the resulting microbiomes happenstances due to the prevalence of microbes as in opportunistic infections that became intimate and functional over time? Once established, are the resulting microbiomes influenced by the selective pressures that interact with the genomes of the plants and microbes? Is the resulting microbiome continued success due to constant genetic mutations occurring among the plants and microbes? Additional questions and potential answers will be addressed in the course of examining what is known about endophytic bacterial and fungal microbiomes that impart or enhance beneficial traits such as disease resistance. Specifically, selective and isolated information will be presented using fungal endophytes (*Epichloë* species), and bacterial endophytes (e.g., *Bacillus mojavensis*) as model microbiome systems. Similarly, other strategies that will be discussed include chemical defenses from herbivory, and the nature of microbe-host signaling, and information on quorum sensing and or anti-quorum sensing compounds, which have evolved to suppress the host response that might have influence over the host's final microbiome load.

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When you drink tequila next time, think in endophytes: cultivable bacteria of *Agave tequilana* and the organic nitrogen transfer

MIGUEL J BELTRAN-GARCIA[1], GLORIA MACEDO[1], AURORA HUERTA[1], ILEANA REYES[1], JAMES WHITE[2], FERNANDA M. PRADO[3], KATIA R. PRIETO[3], MARISA H.G. MEDEIROS[3], PAOLO DI MASCIO[3].

[1] *Departamento de Química, Universidad Autonoma de Guadalajara, Mexico*
[2] *Department of Plant Biology and Pathology, Rutgers University, USA*
[3] *Biochemistry Department, Instituto de Química, Universidade de Sao Paulo, Brazil*

Agaves are plants of importance both in Mexican culture and economy. Mexico is the place of Agave origin, but only *A. tequilana* Weber "blue" variety is permitted for Tequila production. Studies showed that plant-endophyte association stimulate plant growth, improve response against pathogens and abiotic stress and supply organic and mineral nutrients from the rhizosphere and recently, the organic nitrogen obtained by consumption of their own colonizing microbes under nutrient limitation. The biochemical and molecular mechanism has not been fully elucidated, but we have evidence that Reactive Oxygen Species participate on the bacterial degradation and this reinforces the hypothesis of the oxidative nitrogen scavenging (ONS). We analyzed the cultivable microbiome of the "blue agave" and found bacteria belonging to the genera *Pantoea*, *Paenibacillus*, *Klebsiella*, *Pseudomonas*, *Alcaligenes*, *Enterobacter* and *Bacillus* (last two are predominant in seeds), and fungi of the genus *Alternaria* and *Curvularia*. *B. tequilensis* a seed-transmitted endophyte has the ability to colonize the roots and is degraded *in situ*. In this process H_2O_2 is secreted and accumulated in the places with bacterial clusters. To elucidate ONS, we cultured bacteria with $^{15}NH_4Cl$ and these labelled-bacteria was inoculated live or heat-killed to agave plants. The ^{15}N was traced into tryptophan, deoxynucleosides and pheophytin derived from chlorophyll a. The living bacteria promotes plant growth almost three times and increased the ^{15}N content, heat-killed bacteria only provides ^{15}N. We used *E. coli*, as non-endophyte in Agave as control. *E. coli* no induces significant changes in plant growth. Clearly each microbial genus jointly or separately is involved in functions of plant microbiomes. The dissection of the functionality of microbiome components may help us gain a better understanding in how to restore lost microbiomes lost in extensively farmed crops. We will discuss our recent findings on the Agave research and its endophytes.

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Phytohormone signalling and the *Nostoc-Azolla* symbiosis

JAN DE VRIES AND SVEN B. GOULD

Molecular Evolution, Heinrich-Heine-Universitaet Duesseldorf, Universitaetsstr. 1, 40225 Duesseldorf, Germany

Nitrogen is considered a limiting factor in plant growth. For that reason plants enter various symbioses with prokaryotes that have the biochemical capacity to fix atmospheric nitrogen. One very intimate case is the mutualism of the water fern *Azolla filiculoides* and the cyanobacterium *Nostoc azollae*, which the plant accommodates in specialized leaf cavities. What makes this symbiosis unique is the obligate dependence of both on each other and it is the only case known, where a N_2-fixing symbiont is vertically inherited by the next generation of the land plant host. Furthermore, the sophisticated life cycle synchronisation suggests a tight communication between host and symbiont, but our knowledge about the molecular signalling mechanism is sparse. One major group of signalling molecules, not only in plant growth but also in plant-microbe interaction, are phytohormones. Using global gene expression profiling and advanced imaging techniques we analysed the impact of these signalling molecules on plant growth and cyanobiont population. In comparison to angiosperms, we found that auxin and cytokinin had the opposite effect on *Azolla* root meristem development, while showing the same effect on root growth. Cyanobiont density was not influenced by auxin and cytokinin, but other key hormones such as jasmonic acid had a direct effect on cyanobiont density. The *Azolla–Nostoc* symbiosis is a promising system to study how phytohormone signalling, cyanobacterial symbiont population, nitrogen fixation and plant growth are intertwined.

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Transformational approaches in paddy planting: The case of *Trichoderma*-paddy interactions in a biocide-free ecosystem

FEBRI DONI[1], ANIZAN ISAHAK[2], CHE RADZIAH CHE MOHD ZAIN[1], NORELA SULAIMAN[2], NORMAN UPHOFF[3] & WAN MOHTAR WAN YUSOFF[1]

[1]*School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia*
[2]*School of Enviromental Sciences and Natural Resources, Faculty of Science and Technology, Universiti Kebangsaan Malaysia*
[3]*SRI-Rice, Cornell University*

System of Rice Intensification (SRI) is a management approach of rice planting which relies on creating healthy plant growth conditions by minimizing inter-plant competition such as through single planting and wide spacing, and creating healthy soil condition by applying organic amendments, aeration during weeding, and managing water to avoid flooding and water-stress. This management approach results in increase in rice growth and in yield up to three times more than non-SRI cultivation methods. The remarkable enhancement of rice plant growth and yield can be understood in terms of symbiotic interactions between microbes and rice plants in a biocide-free ecosystem. This paper reports our intensive study on the role of a novel isolate *Trichoderma asperellum* SL2 in the enhancement of rice growth and yield under SRI conditions. The research was conducted both in greenhouse (controllable conditions) and paddy fields in Kedah Malaysia (SRI organic certified paddy field). The results showed significant increases in several physiological aspects of the rice plants. Plant growth increased up to 75%, yield increased up to 50%, photosynthesis rate increased up to 50%, stomatal conductance increased up to 50%, the number of stomata increased up to 50%, transpiration decreased up to 50%, water use efficiency increased up to 50%, chlorophyll contents, nutrient uptake and defense enzymes in the paddy significantly increased when colonized with the endophytic fungus. The mechanisms of *Trichoderma asperellum* SL2 in colonizing and improving molecular signaling within plants are also reported in this paper. The overall finding emphasizes that aerobic microbes such as *Trichoderma* have a role in both plant growth regulation and in activation of plant defense mechanisms in paddy, especially under SRI conditions.

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Thank you, may I have another cluster? Horizontal transfer of xenobiotic metabolizing gene clusters among fungal endophytes of maize

ANTHONY E. GLENN

USDA, ARS, Toxicology & Mycotoxin Research Unit, Athens, GA, USA

Maize is one of the world's major commodities, with global production totaling 39 billion bushels in the 2014-2015 season. The plant is well known as a producer of the benzoxazinone and benzoxazolinone phytochemicals (BXs). These are antiherbivory and antimicrobial metabolites also produced by wheat and rye. Some fungi associated with these hosts are able to metabolize the BXs, thus detoxifying them. The corn endophyte *Fusarium verticillioides* is particularly adept at BX biotransformation. The fungus is of significant importance because of its production of the fumonisin mycotoxins, which are a worldwide food safety concern. *F. verticillioides* possesses two gene clusters, FDB1 and FDB2, that are essential for the metabolism of BXs. Phylogenetic and genomic synteny data indicate the two clusters share an evolutionary history, though not driven by gene duplication and divergence, but rather through horizontal gene transfer (HGT) events between fungi commonly occurring on maize. Data suggest *F. verticillioides* acquired the FDB1 cluster via HGT from *Colletotrichum graminicola* with near perfect synteny conservation. Regarding FDB2, a separate and more ancient HGT event is postulated whereby a progenitor *Aspergillus* species also acquired the FDB1 cluster from Colletotrichum. This newly constituted *Aspergillus* cluster, with gene rearrangements, was transferred from *Aspergillus* to an early *Fusarium* species to become the FDB2 cluster, which has been retained to varying degrees by a range of *Fusarium* species. In contrast, *Aspergillus kawachii* is the only extant species of *Aspergillus* known to have a complete FDB2-like cluster. Thus, *F. verticillioides* acquired both clusters from two different fungi by HGT, and the FDB2 genes are themselves derived in part from the FDB1 genes via an earlier, intermediary HGT event involving *Aspergillus*. We hypothesize that host preference for maize and associated exposure to the BX phytochemicals are driving factors in the evolution of these fungal genomes.

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Session: Functional plant microbiomes
Presentation type: Oral
Name, Contact: Haghi Kia Sevda, haghikia@stud.uni-frankfurt.de

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Effect of biotic and abiotic variables on the interaction between plants and fungal endophytes

SEVDA HAGHI KIA[1,2], JOSE G. MACIÁ-VICENTE[1,2]

[1] *Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt, Germany*
[2] *Cluster for Integrative Fungal Research (IPF), Frankfurt am Main, Germany*

Fungal endophytes are known as plant symbionts in most natural ecosystems. Depending on the environmental conditions, they express different symbiotic lifestyles ranging from parasitism to mutualism. Hitherto, our knowledge about the influence of environmental factors on plant-endophyte interactions is sparse. Our aim is to investigate the effect of several biotic and abiotic variables on the symbiosis between root fungal endophytes and plants. For this purpose, we studied 128 fungal endopyhte strains belonging to 60 operational taxonomic units within ten fungal orders, which were isolated from different plants across Europe. To test their effect on hosts' development, all strains were individually inoculated into roots of three plants, namely barley, *Arabidopsis* and *Microthlaspi perfoliatum*, under laboratory conditions. Additionally, we measured several traits of the endophytes—growth rate, sporulation, cellulolytic activity and phosphorus solubilization ability—as potential factors influencing the outcome of their interaction. All tested endophytes were able to colonize plants roots and their effect on hosts varied from neutral to detrimental. The trend and level of the responses were dependent on the plant-isolate combination. Virulence of the strains was correlated with the degree of root colonization by endophytes and fungal growth rate. During the former screening we selected a subgroup of 25 strains for further studies, intended to examine the impact of several abiotic factors (e.g., pH, light intensity or nutrients availability) on the symbiosis. In general there were no significant changes in the interactions along applied gradients of each factor, although in a few cases we could detect a differential response in certain endophytes. Results from this work will allow us to draw hypothesis about the role of plant-endophyte symbioses in natural ecosystems and to define possible factors influencing the position of endophytes on the mutualism–parasitism continuum.

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Symbiotic adaptation: Using endophytes to reprogram plant physiology for agricultural sustainability and food security

RUSTY RODRIGUEZ, LEESA WRIGHT, MALIA MERCER, ELIZA HUDSON, SANG CHO, MELISSA RIENSTRA, MARIANNE HSIEH, ZACHERY GRAY AND REGINA REDMAN

Adaptive Symbiotic Technologies, Seattle, Washington USA

Since the first description of symbiosis in the 1800's, it has become clear that all plants on earth are symbiotic with bacteria and fungi. These microbes can profoundly alter the health and fitness of plants. For example, plants in natural ecosystems adapt to abiotic stress (drought, salinization and temperature) not by genomic DNA modifications, but by establishing symbiotic associations with class II fungal endophytes. Without the appropriate endophyte, native plants are not stress tolerant and do not survive in the habitats to which they are adapted. Over the last 100 years, technological developments in agriculture has included a focus on eradicating microbial pathogens. Unfortunately, this inadvertently resulted in the eradication of beneficial microorganisms. To counter this, several bacterial and fungal products have been developed to improve crop production. These products are focused on improving plant nutrient availability or protecting plants against pathogens, and new efforts are underway to generate additional products. Adaptive Symbiotic Technology has developed the BioEnsure® product line that is based on the ability of fungal endophytes to confer abiotic stress tolerance to crop plants. Symbiotically conferred stress tolerance involves altered plant gene regulation, increased photosynthetic and water-use efficiency, and an increased ability to manage reactive oxygen species. Field tests demonstrate that BioEnsure® increases climate resilience in crops resulting in yield increases of 5-85% depending on the level of abiotic stress.

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Session: Functional plant microbiomes
Presentation type: Poster
Name, Contact: Sujkowska-Rybkowska Marzena, marzena_sujkowska@sggw.pl

Localization of calcium ions in mycorrhizal roots and root nodules of *Medicago truncatula* in response to aluminum stress

MARZENA SUJKOWSKA-RYBKOWSKA

Warsaw University of Life Sciences, Department of Botany

Heavy metals availability to plants can be modulated by soil microorganisms, including *Rhizobium* bacteria and arbuscular mycorrhizal fungi. Both symbioses share similar signaling molecules. The arbuscules and bacteroids are contained within host-derived membranes that represent specialized symbiotic interfaces dedicated to nutrients and signals exchange. Ca_2^+ serves as a messenger in many growth and developmental processes and in plant responses to biotic and abiotic stresses. Numerous signals have been shown to induce transient elevation of cytoplasmic [Ca_2^+] cyt in plants. In the present work, we compare the cellular Ca_2^+ localization in root nodules and mycorrhizal roots of the model legume *Medicago truncatula* grown in presence of aluminum (Al) (50μM for 3h). Calcium was detected in vivo by its reaction with Alizarin S Red, producing red staining in contact with calcium free ions. Al-treated nodules displayed stronger staining in the cell walls and infected cells with highest calcium accumulation in senescent zone compared with control ones. Al-treated mycorrhizal roots revealed stronger alizarin staining at the surface of fungal hyphae and arbuscules compared with control ones. Calcium ions were detected cytochemically by its reaction with potassium pyroantimonate. In both endosymbioses Al treatment induced a rapid hypersensitive response during which highly localised increased accumulation of electron-dense deposits of calcium pyroantimonate were detected. In mycorhhizal roots and nodules numerous precipitates were observed at cytoplasmic side of plasma membrane and around endosymbiont membranes (periarbuscular or peribacteroid), especially around degenerated bacteroids and collapsing arbuscules which where often observed after Al treatment. The precipitates of calcium antimonate were also detected in cytoplasm, vacuoles, near the nuclear envelope and inside karyolymph, especially in cells with degenerated bacteroids or arbuscules. In conclusion, the evidence suggests that changes in calcium homeostasis occurring around endosymbionts membranes may be a major factor in the premature degeneration (stress-induced senescence) of symbiotic compartments under aluminum stress.

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In situ differential response of soil, roots and mycorrhizosphere to altered N/P constraints. Evidence from an N-manipulation experiment in a Mediterranean ecosystem

FLORIAN ULM, CATARINA GOUVEIA, TERESA DIAS, CRISTINA CRUZ

cE3c, FCUL, Campo Grande, Bloco C2, Piso 5 Sala 3, 1749-016 Lisbon, Portugal

Plant acquisition of phosphorus (P) and nitrogen (N) from the soil is mainly dependent on its roots, symbiotic mycorrizal partners and microorganisms in the surrounding rhizosphere. In all cases, the extraction of P and N from organic and inorganic polymers is mediated by extracellular enzyme activities (EEA), which can be used as a functional measure for the turnover rates of these nutrients. EEA are modulated by several factors, including the balance between demand and availability of carbon(C), N and P in the rhizosphere. In an N and P co-limited ecosystem, increasing N availability could alleviate the N limitation but at the same time exacerbate P limitation. As a consequence, this shift should be accompanied by an increase in P-related EEA in relation to N-related EEA, in order to alleviate the P limitation. To test our hypothesis, we used an on-going N-manipulation field experiment in a Mediterranean site (Portugal) that was created in 2007 (Dias *et al.* 2013), because it is known that in this ecosystem plant development is in a general state of co-limitation by N and P. We selected *Cistus ladanifer*, the dominant plant species, which is in symbiosis with ectomycorrhizal fungi (ECM) and sampled its roots (root-tips without ECM), its mycorrhizosphere (root tips colonized with the 4 most abundant root ECM morphotypes) and surrounding soil (rhizosphere). Samples were collected in the autumn and analysed for C/N, nutrient concentration and EEA patterns. Soil nutrient analyses revealed higher soluble N/P ratios in the samples receiving N fertilization, confirming an increased demand for P over N as a consequence of N fertilization. In turn, soluble N/P levels in the soil were correlated with EEA in the soil and the roots, but not the mycorrhizosphere. Interestingly, while the impact of N addition on overall EEA activity in both soil and roots was negative, this effect was not observed in the mycorrhizosphere. The experiment presented here pinpoints to the *in situ* fine-tuning of a functional unit of the ecosystem - the ECM-plant - to altered nutrient constraints, which are a global change driver of great importance.

Dias T, Oakley S, Alarcón-Gutiérrez E, Ziarelli F, Trindade H, Martins-Loução MA, Sheppard LJ, Ostle N, Cruz C (2013): N-driven changes in a plant community affect leaf-litter traits and may delay organic matter decomposition in a Mediterranean maquis. Soil Biology and Biochemistry, 58, 163-171.

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Functions and mechanisms of plant microbiomes

JAMES WHITE, IVELISSE IRIZARRY, MARIUSZ TADYCH, CHRIS ZAMBELL, MONICA TORRES, QIANG CHEN, MARSHALL BERGEN

Department of Plant Biology and Pathology, Rutgers University, New Brunswick, New Jersey USA

Over the past several decades we have come to appreciate that healthy plants host, within and on the surfaces of their tissues, endophytic and epiphytic fungi and bacteria that do not cause disease. Individual components/species of the microbiome of plants have been found to fall into one or more of three major functional groups: 1.) Microbes that alleviate abiotic stress of the host; 2.) Microbes that defend hosts from biotic stress (pathogens and herbivores); and 3.) Microbes that support the host nutritionally, either through increased nitrogen or phosphorus. This functional aspect of plant microbiomes raises the potential to design and construct plant microbiomes for crop plants in order to enhance their cultivation with reduced agrochemical inputs and at lower cost. In order to design and construct functional microbiomes, we must first develop an understanding of the mechanisms by which the microbiome functions. Hypotheses for the abiotic stress tolerance mechanism include: 1.) Oxidative stress protection by increased production of antioxidants produced either by the microbes or hosts in response to microbes; 2.) Ethylene reduction by production of ACC deaminase; and 3.) Ammonia or ammonium detoxification and consequent oxidative stress avoidance. Mechanisms to explain biotic stress resistance generally include production of anti-herbivore or anti-pathogen defensive compounds by the microbe or by the host in response to the microbe (i.e., induced systemic resistance). Mechanisms to explain microbe-mediated enhanced plant growth include: 1.) Stimulation of plant growth due to growth regulator production by microbes; 2.) Increased absorption of nutrients by plants from the rhizosphere due to activities of microbes on roots; and 3.) Increased supply of nitrogen obtained directly from diazotrophic microbes in plants. A close examination of the interactions of the microbe and plant metabolomes may help us to gain a better understanding of the functions and mechanisms of plant microbiomes.

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Discovery, diversity and utilization of endophytes: *Epichloë* species in wild and forage grasses

CAROLYN A. YOUNG

The Samuel Roberts Noble Foundation, Ardmore, OK 73401, USA

Cool season grasses from the subfamily Pooideae are widely distributed, inhabiting many different ecological niches. The success of some grasses is attributed to a dominant systemic fungal symbiotic partner, an endophytic *Epichloë* species (Hypocreales; Clavicipitaceae), which can produce a range of bioactive alkaloids (ergot alkaloids, indole-diterpenes, lolines and peramine) that provide protection to the plant host from herbivory. *Epichloë* species can be sexual (usually nonhybrid) or asexual (hybrid or nonhybrid) and exhibit different transmission strategies (horizontal, vertical or both). Molecular analyses of the symbiont genetic traits among and between host populations allowed us to explore resident endophyte incidence and diversity in host species across multiple grass tribes. *Epichloë* species within a host and between host populations were found to exhibit considerable genotypic and chemotypic diversity of bioactive alkaloid biosynthetic pathways. Variation of alkaloid chemotypes was due to the presence or absence of genes encoding biosynthetic pathway steps. Phylogenetic analyses of intron-rich housekeeping genes (tefA and tubB) and mating-type were used to infer hybrid and nonhybrid origins. Inheritance patterns of the mating-type idiomorphs and alkaloid biosynthesis genes signified hybrid species that have arisen from independent hybridizations of the same ancestral parental species but with different chemotypes, giving rise to added chemotypic diversity. Multiple *Epichloë* taxa or single taxa with multiple chemotypes were found to associate independently with a single host species. Hybrid *Epichloë* species that have been found in diverse hosts are being examined to determine whether they may have jumped between those hosts or, alternatively, arisen from independent hybridization events. The considerable genotypic and chemotypic diversity within the alkaloid biosynthetic pathways and the diverse evolutionary histories identified within the collections provide insight into the broader ecological implications of endophyte-plant symbioses that likely contribute to fitness benefits to the host.

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Using *Trichoderma harzianum* strain TSTh20-1 for revegetation and bio-remediation of oil-contaminated soil

ZAKIA BOUBAKIR[1], TIM REPAS[2], SUSAN KAMINSKYJ[1]

[1] *Department of Biology, University of Saskatchewan*
[2] *Roy NorthernBC, Fort St John*

Soil contamination by petroleum products, gasolines, oils and polyaromatic hydrocarbons (PAHs; collectively GOPAHs) is a major environmental problem. Causes include accidental spills of gasoline and diesel oil at filling stations and refining sites (thousands in Canada alone), which effectively prevent future plant growth. Typically, remediation of GOPAH-contaminated soil begins with physical and/or chemical clean-up. However, these are expensive and only partly restore the soil to a natural state, since the microbiobiota are seldom actively re-established. In situ re-vegetation and GOPAH-bioremediation using a fungal endophyte that both promotes plant growth on PAH-contaminated soil and also grows directly on the GOPAHs themselves, is a promising new technology. Intriguingly, TSTh has been shown to mineralize ^{13}C-phenanthrene to $^{13}CO_2$ under strictly anaerobic conditions, making it ideal for growing in GOPAH-contaminated subsoil. Our recently-patented, systemic, class^{-2} fungal endophyte, *Trichoderma harzianum* strain TSTh20-1 (TSTh) has a suite of desirable characteristics for growth on agricultural as well as GOPAH-contaminated soil. TSTh stimulates seed germination rate and stimulates early root growth, leading to faster seedling establishment. TSTh also increases plant water use efficiency, drought tolerance and drought recovery, leading to higher crop yields. TSTh is already in agricultural field trials in the USA. We will present results on TSTh / tomato seedling growth on soil samples from an oil-spill site in the Canadian Prairies. Future studies will include a temporal analysis of PAH metabolites during degradation under aerobic and anaerobic conditions

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Session Chairs: [1]Karin Pritsch, [2]Thorsten Grams
Chair affiliations: [1]Helmholtz Zentrum Muenchen, Germany
[2]Technische Universitaet Muenchen, Germany

Holobionts as players in ecological stress gradients

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Role of endosymbiotic gut bacteria in the detoxification terpenes by the pine weevil (*Hylobius abietis*)

AILEEN BERASATEGUI[1], KAROLIN AXELSSON[2], OLLE TERENIUS[3], ANNA-KARIN BORG-KARLSON[2], AXEL SCHMIDT[1], JONATHAN GERSHENZON[1], MARTIN KALTENPOTH[1]

[1]*Max Planck Institute for Chemical Ecology, Germany*
[2]*KTH (Royal Institute of Technology), Sweden*
[3]*Swedish University of Agricultural Sciences (SLU), Sweden*

Nutritional symbioses, in which microorganisms provide their host with essential compounds, are widespread in nature. However, nutritional mutualisms are not restricted to the synthesis and provision of essential amino acids or vitamins but also encompass the degradation of noxious compounds in the host's diet. This has been demonstrated in fungal-insect associations but remains poorly described for bacterial-insect associations. The pine weevil (*Hylobius abietis*) feeds on bark and cambium of conifers where it encounters high amounts of terpenoids, a form of conifer chemical defense, that are toxic or deterrent to herbivores. We are interested in studying whether the pine weevil´s gut microbiota is involved in the degradation of such compounds. We have characterized the gut microbial community via next-generation sequencing. We observe a geographically stable microbiota across different European populations dominated by *Wolbachia*, *Enterobacter*iaceae and *Firmicutes*. We have also compared this community to that of other beetles exploiting similar and different ecological niches. Our results demonstrate that the gut microbial community of the pine weevil is similar to that of conifer-exploiting beetles particularly within the *Enterobacter*iaceae family. To explore the functional aspect of this microbial community we have performed bioassays with beetles that have been depleted of bacteria. We observe that bacteria-free individuals do not digest terpenes as efficiently as untreated ones and that supplementation of the native community recovers digestion efficiency. Furthermore, preliminary *in vitro* assays suggest that the gut community can degrade diterpenes. Current experiments with ^{13}C-labelled diterpenes will shed some light on the breakdown products of the digestion of these compounds and bacterial metagenome analyses will provide putative candidates for genes and enzymes involved in such process. Collectively, our results suggest that the bacterial community of the pine weevil is essential for the exploitation of its ecological niche, most likely through the detoxification of plant secondary metabolites.

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1998 - 2014: updating the quantification of symbiotic polychaetes and their hosts

DANIEL MARTIN[1], TEMIR A. BRITAYEV[2]

[1] *Centre d'Estudis Avançats de Blanes (CEAB - CSIC)*
[2] *Laboratory of Ecology and Morphology of Marine Invertebrates (A.N. Severtzov Institute of Ecology & Evolution, RAS)*

The literature on symbiotic polychaetes was reviewed by Martin and Britayev (1998), whose exhaustive analysis included more than 400 references and leaded them to discuss on the characteristics of the symbionts known to date. These were a total of 375 species (292 commensals, 81 parasites) and 969 relationships (713 commensals, 253 parasites) (excluding Myzostomids). As stated by Martin and Britayev (1998), however, "The aim of the review is, therefore, to attract the attention of scientists to - and to encourage further studies on - the ecology of this particular and diverse group of symbionts". Here we demonstrate that this objective was (or is being) accomplished by analysing the new relationships reported since 1998. Currently, the number of symbiotic polychaetes has increased to 580 species involved in 1554 relationships (excluding Myzostomids). Among them, 458 species involved in 1139 relationships are commensals, and 122 involved in 405 relationships are parasites. Since the first know symbiotic polychaete was described by Koch (1846), an average of 2.5 new symbiotic species (2 commensal and 0.6 parasitic) and about 6.6 relationships (4.8 commensal and 1.7 parasitic) were reported per year until 1998. From this year on, the number of newly reported species and relationships has been 12.6 (10.8 commensals, 1.9 parasites) and 49 (40.6 commensals, 8.5 parasites), which overall represents five times more new reports per year for the species and 7.5 for the relationships. Our poster includes two comparative 1998-2014 tables summarizing the symbiotic polychaete families and their host groups, together with their respective relationships. A full list of the symbiotic species and their relationships newly reported since 1998 is also included.

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The microbiome of *Peltigera ponojensis* Gyeln

SERGIO E. FAVERO-LONGO, ENRICA MATTEUCCI, ALESSANDRO DESIRÒ, ROSANNA PIERVITTORI, CINZIA M. BERTEA

[1] *Department of Life Sciences and Systems Biology, University of Torino*

Non-phototrophic bacteria and endolichenic fungi are nowadays considered additional partners of the lichen symbiosis putatively playing functional roles. Biofilm-like bacterial assemblages have been widely described at substrate- and atmosphere-thallus interfaces. By contrast, the occurrence of non-phototrophic bacteria in the photobiont layer has been poorly explored.In this work, the microbiome of *Peltigera ponojensis* was examined. Diffuse colonies of non-phototrophic bacteria were observed by CLSM on the surface of the upper cortex. Moreover, FISH experiments unexpectedly showed endohyphal Alphaproteobacteria in hyphae surrounding the *Nostoc* clusters of the *Peltigera* thalli. TEM observations were also carried out to confirm the occurrence of endohyphal bacteria in the photobiont layer.Isolation of the mycobiont partner was attempted to verify if it was the host of the observed endohyphal bacteria, but without success. By contrast, FISH experiments showed the occurrence of endohyphal Alphaproteobacteria in mycelia of an endolichenic *Cladosporium cladosporioides* s.l. isolated from the photobiont layer, thus recognized as putative fungal host. The finding of endohyphal bacteria within an endolichenic fungal partner adds a novel element to the complexity of the lichen consortium. Recent insights on a functional role of endobacteria in endophytic fungi suggest a potential similar relevance of the observed Alphaproteobacteria for the lichen symbiosis. Moreover, endobacteria may also represent a potential evolutionary driver related to the hypothesized role of endolichenism in the evolution of trophic transition networks in fungi.

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Holobionts as players in ecological stress gradients – Introduction to the session

KARIN PRITSCH[1]*, THORSTEN GRAMS[2]

[1] *Helmholtz Zentrum Muenchen, Germany*
[2] *Technische Universitaet Muenchen, Germany*

Plants and animals do not exist as individuals but live in close association with a plethora of microorganisms, i.e. their microbiome. The resulting complex interactions have been termed ‘holobiont’ representing the typical situation for most eukaryotic organisms. Such holobionts are regarded as the relevant players in ecology and evolution as they shape ecological fitness and as favorable associations are be transferred to the next generation.For interacting plants species, the stress gradient hypothesis predicts the frequency of positive interactions (e.g. nursing or hydraulic lift) to increase in unfavorable environments. Conversely, negative plant-plant interactions are hypothesized to dominate under benign environmental conditions. Transferred to plant or animal holobionts, interaction with their microbiome may be particularly relevant under stressful conditions. Under these conditions plants and animals may profit from the enormous plasticity of their microbial associates represented by the huge genetic potential. Likewise, holobiontic interactions between organs and associated microbes may follow similar principles to alleviate stressful conditions at the organ level. In this way, the holobiont concept opens new perspectives on the plethora of interactions and their resulting performance in the environment.This contribution exemplifies the relevance of holobiontic interactions in plant and animal systems, in particular in response to stressful environmental conditions. Case studies on holobiontic interactions from different organizational levels will shape the focus of the session. Similarities among different organismic associations from the organ to the ecosystem levels will be presented to conclude on the relevance of holobiontic interactions in different environments.

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Concentration and S-isotopic composition of dimethyl sulfur compounds in symbiotic radiolaria: a potentially important unaccounted source for marine DMS

ANDRES GUTIERREZ[1], LOIC PILLET[1], TRISTAN BIARD[1], WARD SAID-AHMAD[2], ALON AMRANI[2], RAFEL SIMO[3], FABRICE NOT[1]

[1] *Plankton group, Diversity and Plankton Interactions Team DIPO, UMR 7144 Station Biologique de Roscoff (CNRS/UPMC) (France).*
[2] *The Institute of Earth Sciences, The Hebrew University, Jerusalem 91904, Israel.*
[3] *Institut de Ciencies del Mar (CSIC)*

Symbiosis with micro-algae (photosymbiosis) is a common feature among marine protists. Yet, very little is known about the physiological mechanisms underpinning photosymbiosis and its significance for pelagic ecosystem structure and function. Dimethylsulfoniopropionate (DMSP) is a widespread metabolite produced by marine microalgae to fulfill multiple eco-physiological functions. DMSP is the precursor of DMS, a climatically active compound that contributes to the formation of cloud condensation nuclei (CCN). Together with organic and sea-salt aerosols, DMS and its sulfur oxidation products have been hypothesized to play a key role in the regulation of earth's albedo and solar radiation. However, the complexity of marine biogeochemistry makes it difficult to unequivocally link the production of biogenic DMS to the formation of sulphate aerosols. Extremely high DMSP intracellular concentrations, for instance, have been recently found in a novel symbiotic association *Radiolaria-Phaeocystis* sp. with worldwide distribution, representing a potentially important and unaccounted source of DMS(P). Using uncultured symbiotic *Radiolaria* as biological model and cutting-edge techniques for sulfur stable isotopes analysis, we conducted a series of experiments aimed at better understanding the contribution of plankton symbiosis to the concentration and S-isotopic composition of DMS(P) in pelagic ecosystems. Here we show that high intracellular concentrations of DMSP are inherent to symbiotic *Radiolaria*. Sulfur isotopic composition of DMSP in the holobiont was significantly higher compared to previously isolated free-living symbiotic microalgae, and marine phytoplankton assemblages, suggesting potentially different biosynthetic pathways associated to photosymbiosis. Incubation experiments conducted using seawater with isotopically distinct sulfate composition allowed us to unveil differences in sulfate uptake and DMSP synthesis between symbiotic *Radiolaria* and free-living microalgae. These results highlight the potentially significant contribution of photosymbiotic plankton to the production and isotopic composition of biogenic DMS(P), with implications for current understanding of sulfur-based aerosols marine sources and sinks.

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Autotrophic and heterotrophic nutrient fluxes in the dinoflagellate-coral symbiosis – A NanoSIMS perspective

THOMAS KRUEGER[1], NOA MASHIAH[2], JULIA BODIN[1], ISABELLE DOMART-COULON[3], MAOZ FINE[2], ANDERS MEIBOM[1]

[1] *Laboratory for Biological Geochemistry, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland*
[2] *The Interuniversity Institute for Marine Sciences, Eilat, Israel*
[3] *Muséum National d'Histoire Naturelle, Paris, France*

The exchange of metabolites between photosynthetic symbionts and their coral host in shallow-water scleractinians provides the nutritional basis for their successful evolution and the existence of massive coral reefs in oligotrophic tropical waters. Autotrophic nutrition, based on carbon- and nitrogen-rich photosynthetic metabolites, is complemented by heterotrophic uptake and assimilation of organic matter to obtain additional carbon, nitrogen and phosphorus. By combining TEM ultrastructural observations with quantitative NanoSIMS isotopic imaging of tissue sections at subcellular resolution, we have compared the exchange of nutrients from the autotrophic assimilation of ^{15}N-nitrate and ^{13}C-bicarbonate with the heterotrophic uptake from ^{15}N- and ^{13}C-labelled brine shrimps in Stylophora pistillata from the Red Sea. Our results confirm that heterotrophy serves as the dominant source of nitrogen for the coral holobiont. On a time scale of 6 hours, heterotrophy provided approximately nine times more nitrogen to the host gastrodermal anabolism than symbiont nitrate fixation and translocation. Heterotrophic nitrogen was also used in anabolic processes in the symbionts, but at a level about 40% lower compared with nitrogen incorporation from nitrate fixation. The role of photosynthesizing symbionts the major contributors of carbon to the coral host was evident from the observation that autotrophic carbon enrichment in the gastrodermal layer was seven times higher in comparison to the heterotrophic carbon acquisition. Our observations directly demonstrate the complementing roles of heterotrophic and autotrophic food acquisition and support the notion that recycling of nutrients in symbiotic coral holobionts is critical to their vitality. The effects of stress from environmental change, including increasing water temperature and ocean acidification, are under investigation.

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Potential effect of metal contamination on the microbiome of Manila clam (*Venerupis phillipinarum*): a culture-based approach

LAURA LEITE[1,2], FLORENCE JUDE-LEMEILLEUR[2], NATALIE RAYMOND[2], FRÉDÉRIC GARABETIAN[2] AND ARTUR ALVES[1]

[1] *Departamento de Biologia, CESAM, Universidade de Aveiro, Portugal*
[2] *Université de Bordeaux UMR 5805 EPOC, Station Marine d'Arcachon, France*

The hologenome theory considers the holobiont (animal or plant and its associated microbiota) as the unit of selection on which evolution acts. One of the assumptions of this theory is that microbial symbionts affect the fitness of the holobiont having an important role in its adaptation, survival, development, health and evolution. Under environmental stress the symbiotic microbial community can change rapidly and therefore assist the holobiont in coping with stress factors. In this study we analysed the diversity of cultivable bacteria associated with Manila clam tissues (mantle, gills, hemolymph) in two non-contaminated sites (Costa Nova, Aveiro, Portugal and Arguin, Arcachon, France) and one metal-contaminated site (Laranjo basin, Aveiro, Portugal). A total of 242 isolates were obtained. These were subjected to molecular typing by BOX-PCR and 196 representatives of the overall diversity identified by 16S rDNA sequencing. The family Vibrionaceae was present in all tissues. Pseudoalteromonadaceae and Micrococacceae were common in mantle, Microbacteriaceae in gills and Rhodobactereaceae in hemolymph. The families common to all study sites were Micrococcaceae, Vibrionaceae, Pseudoalteromonadaceae, Bacillaceae, Pseudomonadaceae and Rhodobacteriaceae. In Arguin and Costa Nova, *Proteobacteria* (mostly Vibrionaceae) were more abundant while in the Laranjo basin *Actinobacteria* (mostly Microbacteriaceae) dominated the microbiota. The results suggest the existence of a core cultivable microbiome in this clam species. Still, it differs according to tissues, revealing that there may exist some tissue-dependence of clam-associated bacteria. Also, metal contamination appears to be influencing the microbiota of Manila clam. It is possible that clams are selecting for *Actinobacteria* partners that are known to be more tolerant to metals and produce useful metabolites, and therefore contribute to the adaptation of clams to this stressful environment as well as their overall fitness. However, this hypothesis needs further testing.

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Session: Holobionts as players in ecological stress gradients
Presentation type: Oral
Name, Contact: Martin Daniel, dani@ceab.csic.es

Comparative phylogeography of two symbiotic dorvilleid polychaetes with contrasting host-crab and bathymetric patterns

PATRICIA LATTIG[1], ISABEL MUÑOZ[2], DANIEL MARTIN[3], PERE ABELLÓ[4], ANNIE MACHORDOM[1]

[1]*Museo Nacional de Ciencias Naturales (MNCN-CSIC), Spain.*
[2]*Instituto Español de Oceanografía, Centro Oceanográfico de Santander (IEO), Spain.*
[3]*Centre d'Estudis Avançats de Blanes (CEAB–CSIC), Spain. 4. Institut de Ciències del Mar (ICM-CSIC)*

Iphitime cuenoti and *Ophryotrocha mediterranea* are two symbiotic polychaetes living in branchial chambers of brachyuran crabs. The former occurs in northern Atlantic and Mediterranean waters from 100 to 600 m depth, inside *Liocarcinus depurator, Macropipus tuberculatus*, and *Bathynectes maravigna*, among others. The latter infests the Mediterranean populations of Geryon longipes, strictly between 600-1200 m depth. According to the potential contemporary genetic breaks and historical events, our study analyses their phylogeographical patterns, as determined by their current genetic diversity based on the mtDNA COI gene. Our results support that I. cuenoti has a weak geographical population structure, with a highly homogenized gene flow between Mediterranean and near Atlantic waters. Populations from Cádiz and Western Alborán showed the highest Fst when compared to the Western Mediterranean ones, indicating a weak barrier effect for Alborán-Oran front. The low host-specificity leading to a wide bathymetric distribution and the vertical swimming capacity of the host apparently contributed to this gene flow homogenization, which is reflected in a star-like haplotype network. In turn, the unimodal haplotype distribution likely indicates a recent expansion, which may be dated after the Pleistocene glaciations.*Ophryotrocha mediterranea* showed a homogenized gene flow, but Eastern Alborán was clearly distinguished from the remaining regions (AMOVA and Fst results). Its haplotype dumbbell-like network and bimodal distribution showed two distinctive lineages apparently not related to current gen flow barriers. At present, they conform a unique metapopulation, which probably suffered a secondary contact as a result of expanding after being isolated, likely during the Pleistocene glaciations. This, together with the low molecular divergence between *O. mediterranea* and *O. geryonicola,* suggest that the former may be a junior synonymy of the latter. However, further studies are required to decide whether there is a barrier separating them or they represent the extremes of a cline.

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Microvirga vignae: a novel legume symbiont adapted to semi-arid soils?

J. LEITE[1], D. FISCHER[2], A. HOFMANN[2], M. SCHLOTER[2], L. ROUWS[3], P.I. FERNANDES-JUNIOR[4], G.R. XAVIER[3], V. RADL[2]

[1] *Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil*
[2] *Helmholtz Zentrum Munchen, Research Unit for Environmental Genomics, München, Germany*
[3] *Embrapa Agrobiologia, Seropédica , Brazil*
[4] *Embrapa Semi-árido, Petrolina , Brazil*

Microvirga vignae is a novel rhizobial species isolated from cowpea nodules. Strains of this genus generally grow well at temperatures above 37°C and tolerate salinity and, hence, could be used for the development of inoculant formulations to improve crop production in semi-arid regions. However, in contrast to other genera of rhizobia, e.g. Bradyrhizobium, which have been extensively investigated, little is known about the ecology and physiology of *Microvirga*. To investigate the distribution of *M. vignae* we screened a collection of 586 isolates obtained from 7 areas of the São Francisco Valley, localized in a semi-arid region of Brazil. The isolates were characterized according to their morphology and 86 strains were selected for genotyping. According to the analyses of 16S rRNA genes only 1 out of the 86 strains was classified as *M. vignae*. To exclude bias from cultivation, we developed primers to detected nifH and nodC genes from *Microvirga* in environmental samples. Those were tested in samples collected from field trials carried out in two arable areas, selected due to their differences in soil texture. Despite of the high sensitivity of the method, we did not detect *M. vignae* sequences in nodules and soils from both areas. Analyses of 16S rRNA amplicons barcode libraries showed that *Microvirga* made only a negligible part of the detected OTUs in the samples. As expected, we observed a clear enrichment of OTUs from the class α-*Proteobacteria* in nodules and rhizosphere compared to soil libraries; many of them were related to Bradyrhizobium sequences. Interestingly, we detected high numbers of OTUs related to *Chryseobacterium*, which was shown to improve nodulation and plant fitness under salt stress. Our results point to a high diversity of putative endophytic bacterial communities associated to cowpea nodules, where *M. vignae* populations might play a minor role.

The evolution of interdependency by neutral evolution in holobionts

MARC-ANDRÉ SELOSSE[1]

[1] *Institut de Systématique, Évolution, Biodiversité (ISYEB - UMR 7205 – CNRS, MNHN, UPMC, EPHE), Muséum national d'Histoire naturelle, Sorbonne Universités, 57 rue Cuvier, CP50, 75005, Paris, France*

In holobionts the evolution of each partner is partly driven by the other partners (co-evolution). The evolution of holobionts is often viewed as a progressive evolution, based on the emergence of new adaptive properties that enhance the fitness, or diversify the niche. In many cases, such evolution enhances the interdependence between partners, because new functions are only achieved in symbiosis. The present paper focuses on the emergence of interdependence, and emphasizes that it can arise even without emergence of any new property, or any progressive evolution. I propose two examples where such a neutral evolution may have acted. First, this may explain why microbiotas are acting both in plant and animals as developmental signals for immunity maturation: the existence of the so-called 'priming effect' was revealed by observation of germ-free animals and non-mycorrhizal plants. Since no evidence supports that microbial signals are more relevant than endogenous ones, a neutral evolution can account for this dependency: any hypothetic endogenous signal can be lost because microbial colonization, reliably occurring at germination or birth, can substitute for it. Second, neutral evolution may explain the extreme genetic reduction in some endosymbionts. Mitochondria and plastids are organelles of endosymbiotic origin, and in some eukaryotic lineages (respectively, anaerobic or achlorophyllous lineages) they fully lost their genomes. Yet, they persisting as bodies surrounded by two membranes dividing in host cytoplasm. This extreme evolution of bacterial dependence to the host is facilitated by the genetic redundancy between the host and the endosymbiotic bacterium. Generally, when two initially independent partners permanently interact, redundant properties become unstable: a mutation in one of the partner can be complemented by the presence of the other, or even by a complementing mutation in the other. Independency is then lost without any gain of function, nor any positive selection. This can thus been viewed as a neutral evolution. Moreover, the accumulation of such ratchet steps over times makes the reversion to independency more and more unlikely: neutral evolution can be predicted to drive reciprocal dependencies within holobionts.

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Bridging ecology and evolution by symbiosis and epigenesis

MICHAEL ELGART, ORLI SNIR, MAOR KNAFO, IRIS AVIEZER, YULIA GNAINSKY, YOAV SOEN

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel

Traditional studies of evolutionary adaptation regard animals and plants mainly as individuals, ignoring the fact that every one of these individual is in fact a community – a partnership between a host and many rapidly reproducing microbes. Consequently, our current understanding of the implications of this structure to adaptation and evolution is very limited.We study these implications experimentally by investigating contributions of gut bacteria in flies to rapid induction and inheritance of new host phenotypes under exposure to novel environments. Conclusions: Our findings show that environmental disruption of the microbiome can increase developmental plasticity, enhance environmentally-induced homeotic transformations, and contribute to non-Mendelian transfer of variation across generations. Significance: These impacts of the symbiotic gut microbiome have profound implications for bridging ecological and evolutionary processes.

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Session Chairs: Manju M Gupta
Chair affiliations: University of Delhi, India

Interdisciplinary approach to mycorrhizal symbiosis

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Session: Interdisciplinary approach to mycorrhizal symbiosis
Presentation type: Poster
Name, Contact: Adriano-Anaya Lourdes, rtdelossantos@gmail.com

P

Differential effect of abiotic factors on Mycorrhization of *Catharanthus roseus* (L.) G. DON in soils of Chiapas, Mexico

M. LOURDES ADRIANO-ANAYA, A. ENRIQUE ALFARO-CORRES, MIGUEL SALVADOR-FIGUEROA, RODOLFO TORRES-DE LOS SANTOS

Centro de biociencias-universidad autónoma de Chiapas, Mexico

Catharanthus roseus (L.) G. Don, or vinca, is a well known plant for its medicinal benefits, such as its anticancer and antiseptic properties (Tembhurne *et al.*, 2012). In the coastal region of Soconusco, Chiapas, Mexico (14 ° 92 ° 16’00 54’00’N ‘W), *C. roseus* grows in a natural way in nutrient-poor soils with high levels of salinity. The arbuscular mycorrhizal fungi (AMF) is related with the tolerance of plants to salinity (Idrees *et al.* 2011). This study evaluated the arbuscular mycorrhizal colonization in 10 samplings of two varieties of *C. roseus* (Alvus and Grape) in different kinds of soils. The AMF colonization was determined according Phillips and Hayman (1979) and Giovannetti and Mosse (1980) with some modifications. Alkaline phosphatase activity (ALP) was determined by Tisserant *et al.* (1993). The salinity and pH analysis was performed according to Guo and Gong (2013). The statistical analysis was carried out by ANOVA with Duncan test $p \leq 0.05$. We found that abiotic factors (salinity, pH and soil location) significantly affected the percentage of root colonization and functionality of simbiosis (ALP). The pH and salinity were the abiotic parameters that most affect mycorrhizal colonization (with a reduction of 25%). In addition, we found no significant differences among genotypes analyzed. The study of the influence of abiotic factors brings evidence on the regulation of mycorrhization in *C. roseus* growing in natural conditions in Chiapas, Mexico, and provide knowledge about the adaptation mechanisms of *C. roseus* to these conditions.

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Resolving phylogeny of indian glomeromycota

ASHA BISHT[1], MEGHA DANDOTIYA[1], MANJU M. GUPTA[2], SHIVANSHU SHARMA[2], MANISHA DOBRIYAL[2], ABHA KUMARI[2]

[1] *Department of Biotechnology, Amity University, Noida, Uttar Pradesh 201303, India*

The aim of this work is to build the phylogeny of 148 species of Glomeromycotean or arbuscular mycorrhizal (AM) fungi known to occur in India. We have analyzed sequence fragment spanning small subunit (SSU), ITS region and large subunit (LSU) nuclear ribosomal DNA. Sequence database has been built for SSU-ITS-LSU region by extracting sequences and sub fragments which covered all three of SSU, LSU and ITS loci together from NCBI genbank. Of the 148 species the desired sequence data is available only for 47 species and represented 339 sequences. Consensus sequences are selected for every species on the basis of percentage identity in multiple sequence alignment (MSA) using Clustal Omega and Phylogeny is built using Phyml with thousand bootstraps. In the present analysis resolution of phylum Glomeromycota to species level using SSU-ITS-LSU as a barcode is found to vary with species studied but it certainly provides a reference data for molecular systematics studies of AM fungi occurring in India.

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A custom fit approach for (cost-)effective use of mycorrhizal fungi

JACQUELINE BAAR

Biomygreen BV, Velp, The Netherlands

Interest in sustainable soil management is rapidly increasing. The application of chemical fertilizers and crop protection agents is reduced due to novel regulations. Therefore, there is a growing interest in the usage of sustainable alternatives such as beneficial soil organisms. In relation to sustainable alternatives, awareness of beneficial mycorrhizal fungal symbionts is rising. As a consequence, interest in the application of mycorrhizal fungal products is growing. Still, application of mycorrhizal fungi can be increased worldwide. Mycorrhizal fungi are often applied in human influenced environmental systems comprising urban, agricultural and natural systems, at least in North-Western Europe. The urban systems, large parts of the agricultural systems and some natural areas, particularly those in our western world, usually have highly disturbed and nutrient-enriched soils. The remaining parts of agricultural systems are often located at soils with low availability of plant nutrients. Considering enhanced usage of mycorrhizal fungi in the human influenced environmental systems, a custom fit approach is needed. Biomygreen has developed such a custom fit approach. This approach is based on the added value of mycorrhizal fungi tailored to the needs of specific plant and tree species, soil environments as well as management regimes. The costs of the mycorrhizal fungi are related to the economic benefits. The custom fit approach is cost-effective and beneficial for the soil ecosystem services. This will be demonstrated with various examples. Furthermore, it will be discussed in which way this custom fit approach can lead to scaling up the application of mycorrhizal fungi and other soil micro-organisms.

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Arbuscular mycorrhizal communities analysis and their effect on growth of Aquilaria crassna and Tectona grandis

AMORNRAT CHAIYASEN[1], J PETER W YOUNG[2], NEUNG TEAUMROONG[3], PAIBOOLYA GAVINLERTVATANA[4], SAISAMORN LUMYONG[1]

[1] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand*
[2] *Department of Biology, University of York, York, UK*
[3] *Schoool of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratch*

Aquilaria crassna Pierre ex Lec. and *Tectona grandis* Linn.f. are sources of resin-suffused agarwood and teak timber, respectively. This study investigated arbuscular mycorrhizal (AM) fungus community structure in roots and rhizosphere soils of both plants from plantations in Thailand to understand whether AM fungal communities present in roots and rhizosphere soils vary with host plant species and study sites. Terminal restriction fragment length polymorphism complemented with clone libraries revealed that AM fungal community composition in *A. crassna* and *T. grandis* were similar. A total of 38 distinct terminal restriction fragments (TRFs) were found, 31 of which were shared between *A. crassna* and *T. grandis*. The estimated average minimum numbers of AM fungal taxa per sample in roots and soils of *T. grandis* were at least 1.89 vs. 2.55, respectively, and those of *A. crassna* were 2.85 vs. 2.33 respectively. The TRFs were attributed to Claroideoglomeraceae, Diversisporaceae, *Gigaspora*ceae and Glomeraceae. Specific AM taxa in roots and soils of *T. grandis* and *A. crassna* were not affected by host plant species and sample source (root vs. soil) but affected by collecting site. Moreover, AM fungal inoculum production from trap culture and utilization to enhance growth of both plants were performed. The *in vitro* inoculation resulted in the regrowth of AM fungal hyphae from initial inoculating root organ culture of *G. intraradices* and *F. mosseae* RYA08, and spore germination of *C. etunicatum* NNT10 and *C. etunicatum* PBT03. *Tectona grandis* plantlets inoculated with *F. mosseae* RYA08 had highest plant height follow by *G. intraradices*, C. etunicatum NNT10, C. etunicatum PBT03, and uninoculated plant, respectively. Inoculation with *G. intraradices* was affected on shoot wet weight but gave low shoot dry weight inferior to *F. mosseae* RYA08. There was no significant different of the number of leave and root wet weight between inoculated and uninoculated plantlets.

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Bioinformatics tools and computer applications for developing insights to understanding arbuscular mycorrhizal symbiosis in India

GUPTA MANJU M

Sri Aurobindo College (University of Delhi) Malviya Nagar,Delhi, INDIA

During last decade our understanding of taxonomy, biodiversity and phylogeny of arbuscular mycorrhizal (AM) symbiosis is much better with development and application of bioinformatics tools. Taxonomic identification of AM fungi from field soil samples is very difficult because the spores collected from field soil samples have lost all the taxonomic characters for identification. Other problems include manual labour as pot culturing that too extends to several months; it requires a lot of expertise and there is a difference of opinion among the scientist regarding consensus classification. Identification of ecological samples has additional difficulties. Recent strides have been made in this area due to two tools: software for molecular barcoding for AM fungal identification and databases and web applications which made identification of field spores closer to possible. These include: MaarjAM summarizes publicly available Glomeromycota DNA sequence data and associated metadata, symGRASS for symbiosis specific genes, AM phylogeny.com for all phylogenetic information and INVAM for culture collection of these fungi, ITS sesquence database for phylogeny resolution and programs like plutoF to input, process, and output the data. Here I present the findings and applications of our database AMF distribution in India – a checklist. We are depicting the species wise distribution and abundance of Indian AM fungal species in 18 states of India along with their consensus classification. It provides a link to the original description of the species and the availability of its culture at CMCC. The barcode sequences SSU-ITS-LSU for many of these species were selected and a consensus phylogenetic tree is shown. Conclusions and significance Our findings highlight the so far unappreciated biological diversity of symbiosis between Glomeromycota and diverse plant roots in India. It provides an analysis of AM fungal species distribution in India apart from a link to consensus sequences and phylogeny information.

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Measuring the benefits of symbiotic rhizobia to legume hosts: Importance of timing, efficiency, and resource hoarding in single- and multi-strain inoculation

KATHERINE E MULLER, ROBERT FORD DENISON

University of Minnesota Department of Plant Biological Sciences

The mutualism between legumes and nitrogen-fixing bacteria known as rhizobia improves yield of legume crops and reduces dependence on chemical fertilizers. We do not know how different traits of rhizobia—such as nodulation timing, resource hoarding, and efficiency of nitrogen fixation—contribute to plant yield and nitrogen status. We also do not know how much increasing the proportion of beneficial rhizobia increases plant benefit. I conducted two pilot experiments to address these questions. The first investigated the relative importance of symbiotic traits by comparing isolates of field-collected soybean rhizobia for nodulation timing, nitrogen fixation efficiency (g shoot mass per g nodule mass), hoarding of the carbon storage compound poly-hydroxybutyrate, and plant benefit measured by biomass and chlorophyll content (a proxy of nitrogen status). The second experiment measured differences in plant benefit and N-fixation efficiency under different ratios of isogenic fixing and non-fixing rhizobia. Two field isolates differed strongly in PHB accumulation, but not in benefits to host plants, measured as biomass and chlorophyll content. There was a weak trend of higher chlorophyll content and biomass with earlier nodulation time, but no differences between isolates. Shoot biomass did not differ between plants inoculated with 90% fixing or 90% nonfixing rhizobia. However, chlorophyll content and nitrogen-fixation efficiency were substantially higher with 90% fixing rhizobia. While these results are preliminary, they suggest that benefits are not substantially different between a small vs. large proportion of high-fixing nodules. Also, the results on PHB accumulation vs. host plant benefit suggest that greater resource hoarding does not necessarily translate to lower plant benefit—though differences in PHB could have been due to differences in plant health that were not discernable with these methods. This line of experimentation will have interesting implications for improving benefits of rhizobia in agriculture and investigating the stability of the legume-rhizobia mutualism.

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Mathematical model for optimal resource allocation in the plant mycorrhizal symbiosis

KOUKI UCHINOMIYA, YOH IWASA

Kyushu University

Many terrestrial plants have mutualistic relation with soil fungi. The plants supply carbon obtained by photosynthesis to the fungi, which in return give a fraction of phosphorus they absorbed from the soil to the plant. Phosphorus is important for the survival and growth of plants especially when they are small. In this study, we focusd on the growth of a small plant (i.e. seedling) growing exponentially. We assumed one plant-one fungus system. They can get more resource when they are grown. When plant gets carbon, plant decides how much allocate it to the fungus. On the other hand, fungus allocates phosphorus to the plant. We first analyse the optimal allocation fraction of resources to the opponent that maximize the speed of the growth of the whole system. As a result, there are 3 types of allocation: allocating all resource, allocating a part of resource and allocating no resource. Allocating all resource is interpreted as investment. By allocating all resource, plant or fungus can encourage the growth of the partner. The partner will give more resource in the future.Next, we derived the optimal resource allocation by plant and fungus to the partner which maximizes the growth speed of the whole system. We assumed the case in which the resource acquisition rates of the plant and the fungus are of Cobb-Douglas type. Then, each player should allocate more to the partner when the resource provided by the partner is more important to its own resource acquisition.

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The ectomycorrhizal status determination of *Phlebopus portentosus* using isotopic analysis

JATURONG KUMLA[1], ERIK A. HOBBIE[2], NAKARIN SUWANNARACH[1], SAISAMORN LUMYONG[1]

[1] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand*
[2] *Earth Systems Research Center, Morse Hall, University of New Hampshire, Durham, NH 03824, USA*

Phlebopus portentosus is one of the most popular wild edible mushrooms in northern Thailand. It can produce sporocarps in the absence of host plant conditions. However, it is still unclear whether *P. portentosus* is a saprotrophic or ectomycorrhizal fungus. *Phlebopus portentosus* spocarps were collected from natural habitat in northern Thailand and identified based on morphological and molecular characteristics. This study aimed to determine the status of this fungus using the stable isotopic analysis and to compare the stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions of *P. portentosus* sporocarps against sporocarps of known saprotrophic and ectomycorrhizal fungi. The result indicated that the average $\delta^{13}C$ of ectomycorrhizal fungi and *P. portentosus* were significantly lower than saprotropic fungi. The average $\delta^{15}N$ of ectomycorrhizal fungi was significantly higher than *P. portentosus,* whereas both were significantly higher than saprotropic fungi. Moreover, the $\delta^{13}C$ and $\delta^{15}N$ of *P. portentosus* samples clustered with ectomycorrhizal fungi and were clearly separated from saprotrophic fungi. Therefore, both stable isotope comparisons indicated that *P. portentosus* in natural habitat is an ectomycorrhizal fungus.

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Cultural characterization of *Pisolithus* species collected from northern Thailand and their *in vitro* mycorrhization with *Eucalyptus camaldulensis* seedling

SAISAMORN LUMYONG, JATURONG KUMLA

Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand

Pisolithus species are the well-known ectomycorrhizal fungi and wide spread in temperate and tropical regions. Basidiomes of *Pisolithus* species were collected from northern Thailand and identified based on morphological and molecular characteristics. The morphological characteristics, spore ornamentation and spore print, distinguished two groups. One group with sharp tipped, erect and crowded spines, and an olive green spore print was identified as *P. albus* and the other with long curved spines, connate narrow cones, blunted, and a brown to dark brown spore print as *P. orientalis* which supported by molecular characteristics. *Pisolithus albus* basidiomes were only collected from *Eucalyptus camaldulensis* plantations, whereas *P. orientalis* basidiomes were collected from *Pinus kesiya*. Pure cultures were isolated from each basidiome and mycelial growth conditions were investigated. The result indicated that both fungal mycelia grew well on modified Melin-Norkans (MMN) agar at 30°C. Fungal mycelium grew on vermiculite/peat supplemented with MMN liquid medium was used as inoculum of the *in vitro* ectomycorrhizal formation between fungi and *E. camaldulensis*. The mycorrhization of *P. albus* and *P. orientalis* were first observed on eucalyptus seedling at three and four months after inoculation, respectively. These results can help select suitable conditions to support mycelial inoculum production for application and management in plant inoculation programs, especially in the inoculation programs of eucalyptus in Thailand.

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Session: Interdisciplinary approach to mycorrhizal symbiosis
Presentation type: Poster
Name, Contact: Oliveira Rui, rsoliveira@uc.pt

P

Seed coating with plant growth promoting microorganisms as an ecotechnological approach for sustainable agriculture

RUI S OLIVEIRA[1,2,3], INÊS ROCHA[1], YING MA[1], ALEŠ LÁTR[4], MIROSLAV VOSÁTKA[5], HELENA FREITAS[1]

[1] *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal*
[2] *CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal*
[3] *Department of Environmental Health, Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Vila Nova de Gaia, Portugal*
[4] *Symbiom Ltd., Lanskroun, Czech Republic*

The dependence of agrochemicals in intensively managed agroecosystems constitutes a worldwide problem with environmental, economic and social consequences. There is, thus, an increasing need to find alternatives to intensive agriculture. Plant growth promoting soil microorganisms such as plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) may reduce the need for chemical input, while improving plant growth and yield. Currently, there are no feasible technologies for application of AMF and PGPR in large scale agriculture. The seed coating technique has the potential to allow the use of minor amounts of inoculum, resulting in cost reduction and efficiency increase. The aim of this study was to reduce the application of chemical fertiliser by using beneficial microorganisms inoculated via seed coating. Seeds of common wheat (*Triticum aestivum*) were coated with inoculum of *Rhizophagus intraradices* BEG140, *Pseudomonas fluorescens* F113 or a mixture of both microorganisms and germinated in agricultural soil under controlled conditions. Plants were grown under different regimes, ranging from no input to high input of chemical fertiliser. Results showed that both the application of chemical fertiliser and microbial inoculation influenced plant growth. Plants inoculated with AMF via seed coating were successfully colonised. Seed coating may represent a viable technique for large scale application of beneficial microorganisms, while reducing the input of agrochemicals.

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Molecular characterization and genetic diversity analysis of ectomycorrhizal fungi associated with *Quercus leucotrichophora* (Oak)

VEENA PANDE, AMIT PANWAR

Department of Biotechnology, Kumaun University, Bhimtal Campus, Bhimtal-263136, India

In ecological settings, oak are functionally obligately associated with a diversity of ectomycorrhizal (ECM) fungi and benefit from the symbiosis in term of growth, seedling establishment and survival. Identification of Ectomycorrhiza Fungi (ECM) based on morphological characters has been laborious and time consuming, especially samples collected from the environment. Additionally, due to its microscopic nature and limited morphological characters, intraspecies variation is difficult to detect. In the present study, the genetic diversity and molecular characterization of ectomycorrhizal fungi from different forest sites of Kumaun region of Uttarakhand, predominated by *Quercus leucotrichophora* (oak) species and varying with respect to altitude, aspect, age and composition of forests was done. In total, 50 ectomycorrihzal species were collected comprising 19 genera. Mycelia of 15 species of ectomycorrhizal fungi representing genus *Russula* were isolated in pure culture and characterized by molecular methods. Molecular identification and Inter specific variation among these species were evaluated by analyzing the internal transcribed spacer (ITS) of the rDNA region using restriction fragment length polymorphism (RFLP). Depending on the restriction profiling, the closely related species could be grouped on the basis of common fragment patterns, thereby confirming the potential of the ITS region in PCR-RFLP in molecular characterization and diversity assessment of ectomycorrhizal fungi. Based on the result of the present study the potential of ITS region PCR-RFLP analysis is recommended for characterization, identification and diversity assessment of ectomycorrhizal fungi associated with oak.

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Application of arbuscular mycorrhizal fungi inoculum through seed coating for large scale agriculture

INÊS ROCHA[1], YING MA[1], ALEŠ LÁTR[2], MIROSLAV VOSÁTKA[3], HELENA FREITAS[1], RUI S. OLIVEIRA[1,4,5]

[1] *Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, Coimbra, Portugal*
[2] *Symbiom Ltd., Lanskroun, Czech Republic*
[3] *Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic*
[4] *CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal*
[5] *Department of Environmental Health, Research Centre on Health and Environment, School of Allied Health Sciences, Polytechnic Institute of Porto, Vila Nova de Gaia, Portugal*

The application of arbuscular mycorrhizal fungi (AMF) in agriculture has become of great interest due to their potential roles in sustainable crop production. Nevertheless, the inoculation of AMF by broadcasting in large scale agricultural fields is not feasible because non-targeted spreading of inoculum over large areas results in high cost per plant. Seed coating has the potential to reduce the amount of inoculum needed, resulting in cost reduction and efficiency increase. The aim of this study was to assess whether seed coating with AMF inoculum is a feasible delivery system for large scale agriculture. Maize seeds were coated with inoculum of Rhizophagus intraradices BEG140 and germinated in agricultural soil under controlled conditions. A treatment using uncoated seeds with direct soil inoculation of *R. intraradices* was included as positive control. Plants had percentages of root length colonised higher than 60% either after direct soil inoculation or with coated seeds, with no significant differences between the treatments. Results indicate that seed coating can be used to apply lower amounts of inoculum. Seed coating is an innovative approach for large scale application of AMF, which may result in economic and environmental benefits.

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The lifestyle of arbuscular mycorrhizal fungi in the light of genomics

CHRISTOPHE ROUX[1,2]

[1] *Université de Toulouse, UPS, UMR5546, Laboratoire de recherche en Sciences Végétales, BP42617, F-31326 Castanet-Tolosan CEDEX, France*
[2]CNRS, UMR5546, BP 42617, F-31326 Castanet-Tolosan CEDEX, France

Arbuscular Mycorrhizal (AM) symbiosis is considered as the most widespread mutualistic plant-fungal association. AM fungi (Glomeromycota) can indeed associate with non-vascular plants (liverworts, hornworts), early diverging vascular plants (ferns) or seed plants. Such broad host spectrum is unique among plant interacting fungi. Previous works in comparative phylogenomics showed that mycotrophic plants -i.e. plants hosting AM fungi- share conserved genes that seem required for AM symbiosis. By contrast the AM fungal “symbiotic gene toolkit” remained poorly characterized, mostly due to the absence of related genetic tools. This is why the recent release of genome assemblies from the AM fungus Rhizophagus irregularis strain DAOM197198 (Tisserant, Malbreil *et al.*, 2013; Lin, Limpens *et al.*, 2014) represents an important step forward in the study of AM fungal biology. In contrast with previous works suggesting a heterokaryotic organisation of AM fungi (different nuclei in each individuals), DAOM197198 is homokaryotic with a haploid genome of ca 154 Mb. As expected for such large fungal genome, transposable elements are strongly represented, at least one third of the genome. Ongoing works deals with the dynamics of TE activity and their incidence on genome plasticity. Based on the designed gene repertoires, we compared the transcriptomes of two AM fungal species, *Rhizophagus irregularis* and *Gigaspora rosea*, in association with flowering plants (monocot and dicot) and a liverwort. Interestingly, the obtained gene patterns - including nutrient transporters, metabolic genes and a panel of secreted proteins potentially involved in host manipulation- were highly conserved whatever the host. How these genes can be considered as remnant of the ancient AM fungal symbiotic gene network will be discussed.

Tisserant, Malbreil *et al.* (2013): *PNAS 110*:20117-22
Lin, Limpens *et al.* (2014): *Plos Genet 10*:e1004078

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Contribution of native AM fungi to soil carbon sequestration assessed in the form of glomalin and C-stocks in different soil and crop management practices of soybean-based cropping system

RICHA AGNIHOTRI, ASHU PANDEY, A RAMESH, SD BILLORE, MAHAVEER P SHARMA

Indian Council of Agricultural Research- Directorate of Soybean Research, DARE, Govt of India, Indore-452 001, India

Soils have the potential to mitigate CO_2 emission by sequestering soil carbon through changed crop and soil management practices with biological interventions. The role of arbuscular mycorrhizal (AM) fungi in plant production systems and carbon cycling has been widely recognized. Arbuscular Mycorrhizal fungi are obligate biotrophs which have been known to make significant contribution to soil carbon sequestration. These fungi through production of glomalin and their hyphal network maintain stable C-stocks within soil while improving overall soil structure. In the present study the impact of AM fungi was assessed in two ongoing field trials viz., in trial 1 native AM fungi (*Glomus intraradices*) was applied in sole soybean, sole maize and soybean + maize grown in conventional and organic farming practices and in the second trial which was a long term field trial where soybean- wheat and soybean-chickpea were taken in organic, inorganic and integrated farming practices. The AMF biomass, glomalin production, microbial biomass carbon and carbon stocks were assessed. It was observed that in the first trial, the AM inoculation enhances the carbon stocks and glomalin in the plots applied with organic manure under soybean + maize cropping system (18.95 Mg C/ha/year). On the other hand in the second trial the AM fungi biomass and glomalin production (formed due to background resident AM population) were found to be higher in soybean-chickpea system taken under organic practices and maintained higher carbon stocks (22.68 Mg/C/ha/yr) and glomalin (0.52 mg GRSP/g soil) and higher microbial biomass carbon. The findings concluded that AMF inoculation under first trial and building up of AMF biomass in long term field trial influenced due to crop and soil management practices which eventually enhanced the glomalin production ultimately contributed towards the higher carbon stocks in soil.

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Comprehensive analysis of pea (*Pisum sativum* L.) mutants with defects in both arbuscular mycorrhiza and nodule development

OKSANA Y. SHTARK[1], YAROSLAVA V. FEDORINA[1], DARYA M. SULGINA[1,2], ALEKSANDR I. ZHERNAKOV[1], ALEKSEY A. KRYUKOV[1], VLADIMIR A. ZHUKOV[1], & IGOR A. TIKHONOVICH[1]

[1] *All-Russia Research Institute for Agricultural Microbiology (ARRIAM), Podbelsky chaussee 3, 196608, St. Petersburg, Pushkin (Russia).*
[2] *Saint Petersburg State Institute of Technology (Technical University), Moskovsky prospect, 26, 190013, St. Petersburg*

Pea (*Pisum sativum* L.), the important food crop, forms both arbuscular mycorrhiza and nitrogen-fixing nodules. To study genetic control of the symbioses development in pea, we currently use an approach that combines detailed phenotypic analysis of pea mutants from the extensive ARRIAM collection, gene mapping with the use of both morphologic and molecular interspecies markers, sequencing of candidate genes found after comparison of resulting pea genetic maps with *Medicago truncatula* genome and/or mutant phenotypes, and recently, analysis of our own assembly of pea total symbiosis-specific RNA-Seq data. We have analyzed mycorrhizal phenotype of 12 nodulation (Nod– or Fix–) pea mutants in 11 Sym genes after 10 and 20 days of cultivation in nurse-plant systems with Rhizophagus irregularis. The development of external mycelium (EM%), internal mycelium (M%), and arbuscules (A%) was studied. Mutations in Sym7 (NSP2 homolog), Sym11, and Sym14 genes resulted in 2-4 times increase of EM% compared to the wt. The sym11 mutant was almost non-mycorrhizal; sym7 and sym14 had reduced M%, but only sym7 showed reduced A%. The sym34 had more than tenfold reduced both EM% and M% at 10 days. The sym27 had twofold reduced both EM% and M%, but normal A%. The sym23, sym24, and sym26 demonstrated only reduced A% at 10 days. The sym5, sym32, and sym38 did not differ from the wt. The *M. truncatula* homologues for two genes have been revealed: NSP1 for Sym34, and Vapyrin for Sym36 (the mutant phenotype described earlier is: Nod–; stunted arbuscules). Some analyzed genes (Sym5, Sym14, Sym27, Sym32, Sym36, and Sym38) were precisely mapped in pea genome. Both mapping and candidate genes examination are continued. Thus, our work based on comprehensive analysis of pea mutants allows not only identifying new symbiotic pea genes and their homologs, but also suggests new functions of the known genes.

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Chair affiliations: [1]Florida Gulf Coast University, USA
[2]University of Graz, Austria

Lichen symbionts:
Marriage, divorce, and domestic partnership

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Ontogeny of cyanobacterial cephalodia and non-photosynthetic symbiotic bacteria in the lichen *Lobaria virens* (Whith.) J.R. Laundon

FRANCISCO GARCÍA-BREIJO[1], JOSÉ REIG-ARMIÑANA[2], ARANTXA MOLINS[2], NURIA CEBRIÁN[2], EVA BARRENO[2]

[1] *Dpto. Ecosistemas Agroforestales, Universidad Politecnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.*
[2] *Instituto ICBIBE, Dpto. Botanica, Jardi Botanic, Fac. C. Biologicas, C/ Dr. Moliner 50. 46100-Burjassot, Valencia, Spain*

Lobaria virens is a well-known tripartite lichen (mycobiont, phycobiont, cyanobiont) growing on the tree bark of old, well preserved forests which have not suffered from the impact of pollutant agents (ecological continuity). The European Red-lists of endangered lichens consider that it is a species which is highly sensitive to small environmental changes and, nowadays, in Central and Northern Europe it is said to be in a critical state, or extinct. Surprisingly, the anatomical, ultrastructural or molecular features of this interesting taxon have been poorly studied. Moreover, in the literature only two allusions to the internal cephalodia can be found, but this is not even in the original description. In Asturias (N of Spain) it is not difficult to find forests around small rivers of non-polluted water which harbour healthy populations of this lichen. We collected fresh material there and studied the ontogenetic development of cephalodia, pycnidia and apothecia by LM, SEM and TEM techniques, as well as the molecular characterization of three of the symbionts. In this work we present the complete ontogeny of the cephalodia, which is similar to that of the North American Lobaria quercizans, pycnidial and apothecial structures. The nrITS phycobiont matches with Dictyochloropsis reticulata, and we also provide ultrastructural TEM images. The 16S and TrnL sequences clearly identify the cyanobacteria as Noctoc sp. Furthermore, TEM images show the ultrastructure of this *Nostoc* in symbiosis.Unexpectedly, in the upper cortex we found non-photosynthetic bacterial colonies in close contact with living hyphae with a different shape to those of the cortical paraplectenchyma. These bacteria are able to enter due to the fungus which seems to start up special channels where the bacteria may migrate inside the thallus. How these ontogenetic processes can influence thalli symbiogenesis is an open question (MINECO, CGL2012-40058-C02-01; FEDER; GVA, PROMETEOII/2013/021).

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Metabolic profiling of alpine and Ecuadorian lichens

VERENA K. MITTERMEIER[1], NICOLA SCHMITT[1], LUKAS VOLK[1], JUAN PABLO SUÁREZ[2], ANDREAS BECK[3,4], AND WOLFGANG EISENREICH[1]

[1] *Lehrstuhl fuer Biochemie, TUM, 85747 Garching, Germany*
[2] *Departamento de Ciencias Naturales, Universidad Técnica Particular de Loja, San Cayetano Alto s/n. Loja, Ecuador*
[3] *Botanische Staatssammlung Muenchen, 80638 Muenchen, Germany*
[4] *GeoBio-Center, LM*

Non-targeted 1H-NMR and GC-MS methods were used to analyze crude extracts from several alpine and Ecuadorian lichens collected from their natural habitats. In control experiments, the reproducibility of metabolite detection was tested with polar and apolar extracts from *Stereocaulon* alpinum retrieved from different locations or during different annual and seasonal periods. A comparison of the data obtained from the alpine and Ecuadorian lichens under study revealed genus- and species-specific profiles. The discriminant functions determining cluster formation in principle component analyses (PCA) were due to ubiquitous metabolites such as trehalose or ornithine, but also due to lichen-specific metabolites such as sticticin in *Sticta* species. Varying amounts of sugars and sugar alcohols in the same lichen species e.g. due to different environmental conditions during collection appeared of minor relevance. The metabolic clusters matched phylo-genetic analyses using nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences of lichen mycobionts, as exemplified with the genus *Sticta*. It can be concluded that NMR- and/or GC-MS-based non-targeted methods are useful additional tools in the chemo-taxonomy of lichens that could also benefit the discovery of novel lichen metabolites on a rapid and systematical basis.

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The potential of resistance of the lichen symbiosis to extraterrestrial conditions: Exposure to space and Mars-analogue conditions on the ISS and exposure to simulated galactic cosmic rays

ANNETTE BRANDT[1], JOACHIM MEESSEN[1], MARINA RAGUSE[2], JEAN-PIERRE DE VERA[3], SILVANO ONOFRI[4], RYUICHI OKAYASU[5], RALF MÖLLER[2], SIEGLINDE OTT[1] AND HIMAC TEAM[5]

[1] *Institute of Botany, Heinrich-Heine University, Düsseldorf*
[2] *Institute of Aerospace Medicine, Cologne*
[3] *Institute of Planetary Research, Berlin*
[4] *Department of Ecological and Biological Sciences, Tuscia University, Viterbo*
[5] *NIRS, Chiba*

The lichen symbiosis between a fungus (mycobiont) and an alga/cyanobacterium (photobiont) can be regarded as a successful life strategy, enabling the colonization particularly of severe and extreme habitats.The resistance of lichens to harsh environmental parameters stimulated the investigation of their viability under even more hostile, extraterrestrial conditions. Samples of *Xanthoria elegans* were exposed for 1.5 years to space outside the International Space Station (ISS). This first long-term exposure of eukaryotic organisms tested their ability to cope with vacuum, rapid temperature cycling and insolation of accumulated doses ≤4.9 GJm−2 including ≤0.3 GJm−2 of UV irradiation and ≤0.2 Gy of cosmic radiation. A Mars-analogue sub-set of samples was used to check the organisms' ability to resist Mars-like environmental conditions. The samples demonstrated considerable resistance to the applied conditions. The photobiont resumed photosynthetic and cell viability. The analyses indicated 50-80% active algal cells and 60-90% active fungal hyphae. The viability was confirmed by culture experiments, demonstrating algal growth after any of the ISS exposure conditions. Ultrastructural analyses revealed impairment on the photobionts' cellular structure by long-term space vacuum and re-inforced desiccation. The analyses of the ISS exposure experiment lead to the conclusion that not the UV-radiation, but space vacuum was the most important deleterious stressor to the anhydrobiotic lichen samples. To assess the lichens' resistance to ionizing radiation, it was irradiated at high doses with four different qualities of ionizing radiation (≤112 kGy) under laboratory conditions, resembling the Galactic Cosmic Rays beyond the magnetic shielding of Earth. Additionally, X-ray irradiation experiments were performed with hydrated, metabolically active and photosynthesizing *Xanthoria elegans* samples, with and without the natural rock substrate (≤100 Gy). The study with its results is intended to assess the potential of the symbiotic *Xanthoria elegans* to resist deep space radiation climate.

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The lichen *Thamnolia vermicularis*, a lonely fungus with many green friends

IOANA ONUT BRÄNNSTRÖM, HANNA JOHANNESSON

Uppsala University, Sweden

The process of lichenization enables two habitat-dependent organisms, the fungi and the algae, to survive in environments hostile for its partners when isolated. The cosmopolitan, alpine and arctic lichen *Thamnolia vermicularis* represents one example of a successful colonizer. Nevertheless the assumed lichen spreading biology by fragments and the fact that its fungal partner seems to completely lack sexual reproduction the species huge geographic range is puzzling. We were able to confirm the species asexuality by sequencing the genomes and transcriptome of the fungal partner, and investigating the distribution of mating types in natural populations. First, we used the architecture of the mating-type idiomorphs to gain insight into the mating systems. The fungal genome harbors mating-type idiomorphs consistent with a heterothallic (i.e. self-incompatible) mating system. When screening a sample of 218 individuals from natural populations of *T. vermicularis*, we find only one of the mating types suggesting that sexual reproduction is constrained by the existence of a single mating type in natural populations. Even if fragment dispersal as holobiont can be advantageous in the short term, asexual lineages are considered evolutionary dead-ends. We hypothesize that the lichenized fungus of *T. vermicularis* adapt to a changing environment by associating with different algal species in different localities. If these algal haplotypes are adapted to a certain environment the switching to different photobionts might play a role by enabling the lichenized fungus to extend its geographic range and conquer new environments. Our data suggests that this might indeed be the case for the fungus of *T. vermicularis*. Using NGS sequencing data we discovered that on nutrient poor soils of Iceland, it shares the photobiont with two other genetically distant lichenized fungal species *Cetraria islandica* and *Cetraria aculeata*.

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Ramalina farinacea symbionts: marriage, polygamy and predominance

MYRIAM CATALÁ[1], EVA BARRENO[2]

[1] *Dep. Biología y Geología, Física y Química Inorgánica. ESCET. Universidad Rey Juan Carlos, E-28933 Móstoles, Madrid, Spain.*
[2] *Instituto Cavanilles de Biodiversidad y Biología Evolutiva. Dpto. Botánica, F. CC. Biológicas. Dr. Moliner 50. E46100*

This intrinsic diversity in lichens results in a huge taxonomic and ecophysiological diversity. In the holobiont thallus, the positive interactions between the partners generate a synergy that allows the adaptation to anhydrobiosis and the colonization of harsh and extreme habitats albeit the mechanisms are unknown. A ROS burst accompanies rehydration, contributed mainly by photobionts' photo-machinery. We reported the presence of two phycobionts, *Trebouxia* TR1 and T. TR9, with different ultrastructural features and physiological responses against oxidative stress within the same thalli of *Ramalina farinacea*. Our recent results indicate that the oxidative response of the entire thallus during rehydration is modulated by the predominant microalga. This can have important ecophysiological and adaptive implications since thalli components proportion may vary among populations. We formerly described that while TR1 puts up an expensive constitutive response against Pb-elicited oxidative stress, TR9 develops an intense inducible response leading to a quantitatively similar response. Anhydrobiosis mechanisms are intimately related with general mechanisms of tolerance to abiotic stress (i.e. hypersaline media, heavy metals). All these processes involve an excellent modulation of oxidative stress, in which, according to our results, NO plays a relevant role. Mycobiont-released NO seems critical in the photoprotection of *R. farinacea* during rehydration despite phycobionts are able to produce their own supply. The reasons underlying this mycobiont-NO dependence are not known and might vary according to the predominant phycobiont due to their different behavior when NO is inhibited during rehydration of axenic cultures. Some other observations of our "Lichen Symbiogenesis Team" seem to support a model where ecological diversification and speciation of lichen symbionts is likely to be promoted by different physiological backgrounds of several phycobionts in individual thalli. We conclude that our findings reveal new perspectives on the adaptive mechanisms of lichens to provide such biological success.

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Exploring the microbiome of the optionally lichenized fungus *Schyzoxylon albescens*

SAMANTHA FERNÁNDEZ BRIME[1], LUCIA MUGGIA[2,3], MARTIN GRUBE[3], MATS WEDIN[1]

[1] *Swedish Museum of Natural History, Dept. of Botany, Stockholm, Sweden*
[2] *University of Trieste, Dept. of Life Science, Trieste, Italy*
[3] *Karl-Franzens University of Graz, Institute of Plant Science, Graz, Austria*

Lichens are traditionally viewed as integrated associations between a fungus (mycobiont) and photosynthesizing organisms (green algae and/or cyanobacteria) that provide the fungus with carbohydrates. In addition, recent studies have shown that nonphotosynthetic bacteria may form abundant, diverse and presumably lichen-species specific bacterial communities inside the lichen thallus. These new discoveries have challenged the traditional view of the lichen symbiosis and stressed that lichens should be viewed as mini ecosystems, where microbial communities may contribute to and facilitate the lichenization process, but where the role of the participating bacteria still is very unclear. Here, we will explore the microbial communities of *Schizoxylon albescens*, (Stictidaceae, Ostropales, Ascomycota). The Stictidaceae contains saprotrophic, parasitic and lichenized fungi, and several species that can live either as saprotrophs or as lichens depending on the substrate they grow on, a phenomenon termed “optional lichenization”. *Schizoxylon albescens* either grows as loosely lichenized on *Populus* bark or as a saprotroph on dead *Populus* branches. In order to study the microbial diversity of this species, we used molecular data generated on the Illumina MySeq sequencer combined with microscopic studies using DNA fluorescence *in situ* hybridization. Our study aims at increasing our knowledge of the microbial diversity in lichens, and especially focusses on finding if specific bacteria are associated to the lichenized and saprotroph morphs that could potentially have unique roles in their different nutrition lifestyles. We expect to improve our understanding of the lichenization process, of optional life-styles in fungi, and towards developing a “model” fungus for experimental studies on interactions including lichenization.

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Presentation type: Poster
Name, Contact: Hinojosa-Vidal Ernesto, eva.barreno@uv.es

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Phylogenetic analysis of symbiotic *Trebouxia* microalgae found in sorediate lichens of *Parmelia* reveal new monophyletic clades

ERNESTO HINOJOSA-VIDAL[1], FERNANDO MARTÍNEZ-ALBEROLA[1], FRANCISCO GARCÍA-BREIJO[2], JOSÉ REIG-ARMIÑANA[1], EVA BARRENO[1]

[1] *ICBIBE, Dpto. Botánica, Jardí Botànic, Fac. C. Biológicas, C Dr. Moliner 50. 46100 Burjassot, Valencia, Spain.*
[2] *Dpto. Ecosistemas Agroforestales, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain*

Parmelia is a genus of medium to large foliose lichens widely distributed across the world. Although phylogenetic relations within lichen-forming fungus *Parmelia* have been broadly studied and well stablished, little is known of their chlorobiont diversity. Previous studies seem to point to unicellular green algae *Trebouxia* as the preferred phycobiont within the genus. However, as stated before, lack of molecular and morphological data from these algae seems to be a weakness in the understanding of the biodiversity and ecological variability within the genus. We aim to provide a new insight into the phylogenetic relationships among the different microalgae found in *Parmelia* selected individuals from the sorediate and closely related *Parmelia sulcata* Taylor and *P. barrenoae* Divakar, Mc. Molina & A. Crespo, were collected across the Iberian Peninsula. DNA extracts from both fungi and microalgae were analysed by molecular techniques. Nuclear nrITS and chloroplastic LSU rRNA genes were chosen for the phycobionts and concatenated after their evolutionary models were proven the same with jModelTest. The barcode nrITS gene was selected for the mycobionts. Morphological and ultrastructural studies were performed in the whole symbiotic thalli as well as in several isolated and cultured microalgae.Phylogenetic analysis reveal what appears to be new *Trebouxia* clades present among the analysed microalgae that do not provide a match with any gene sequence available at the GenBank database nor described in any other publication. One clade, not clearly related to any other *Trebouxia*, appears as an exclusive symbiont in *Parmelia sulcata*. Our data also suggest a new clade of *Trebouxia* associated to *Parmelia barrenoae* and seemingly related to any other *Trebouxia*, appears as a exclusive symbiont in *Parmelia sulcata*. Our data also suggest a new clade of *Trebouxia* associated to *Parmelia barrenoae* and seemingly related to the *T. impressa* complex.

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Reconstruction of fossil lichen communities: from molecules to palaeoecosystems

ULLA KAASALAINEN[1], CHRISTOPH HARTL[1], NADINE SCHÄFER[1], JOCHEN HEINRICHS[2], JOUKO RIKKINEN[3], AND ALEXANDER R. SCHMIDT[1]

[1] *Department of Geobiology, University of Göttingen, Germany*
[2] *Systematic Botany and Mycology, University of Munich (LMU), Germany*
[3] *Department of Biosciences, University of Helsinki, Finland*

Amber is fossilized resin of gymnosperm and angiosperm trees and it is famous for its lifelike preservation of arthropods, plant remains, and microorganisms. The fossil record of many lineages of fungi, plants, and arthropods is actually restricted to amber inclusions and even the preservation of soft-bodied microorganisms has cellular and ultrastructural fidelity. Amber is an ideal medium for palaeoecosystem research since it is still organic but long-lasting and it preserves even chemical features of the plant from which the resin originated together with data about the palaeoecosystem including a plethora of entrapped organisms from the same habitat. Since many lichen species grow as epiphytes on tree trunks, also lichens occur as fossils entrapped in amber. The analysis of amber inclusions will consequently open a window to ancient lichen communities and has the potential to answer general questions addressing the evolution of lichen symbioses through earth history. However, even though lichens are best preserved in amber, only few specimens so far have been found and described.Our study focuses on lichen symbioses in the Paleogene, 55-23 million years before present, and in the evolution of these symbioses until the present. In addition to macromorphological features, the identification of the fossil lichens is based on ultrastructural features and on chemical analyses of organic molecules. These methods include for example scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDX), and Raman spectroscopy. The available material and methods permit for the first time a large-scale reconstruction of fossil lichen communities including interactions, e.g. with associated fungi, arthropods, and plants. In addition, the Paleogene lichens and lichen communities are compared to their extant relatives in order to track and analyze the essential evolutionary changes in community structure, photobionts, ecology, and secondary chemistry.

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Photobiont mediated guild structure of epiphytic cyanolichens

ULLA KAASALAINEN[1,2], VEERA TUOVINEN[1], AND JOUKO RIKKINEN[1]

[1] *Department of Biosciences, University of Helsinki, Finland*
[2] *Department of Geobiology, University of Göttingen, Germany*

Most lichen-forming fungi are highly specific in their choice of symbiotic cyanobacterial strains. Most cyanobionts of Lecanoromycetes (Ascomycota), the largest class of lichen-forming fungi, belong to the genus *Nostoc* (*Nosto*caceae, *Nosto*cales). In addition, certain fungal genera, like *Coccocarpia* (Coccocarpiaceae), associate with another recently characterized nostocalean genus *Rhizonema*. Lichens reproduce and disperse either by producing symbiotic propagules or sexually by producing fungal ascospores. After dispersal the germinating ascospores must establish a new association with a compatible cyanobiont. The availability of compatible cyanobacterial strains can limit the establishment success of the spores. It has been suggested that the germinating fungal spores may obtain compatible cyanobionts from pre-existing thalli of the same or some other cyanolichen species. Such local assemblages of lichen species depending on the same cyanobiont strain could significantly improve their mutual success by sharing compatible cyanobionts on the community scale.Here we present a comprehensive analysis of symbiont specificity and photobiont sharing among epiphytic cyanolichens. We studied representatives from over ten peltigeralean genera associated with two nostocalean cyanobacterial genera. The results revealed the vast diversity of lichen forming fungi and the associated cyanobionts, and the unprecedented complexity of the association networks shared by the representatives of several lichen genera. This, so called guild structure, is mediated via shared symbiotic cyanobacterial strains, and it may be essential for the presence and survival of ecologically more diverse lichen taxa. The structure and principals of the photobiont mediated guilds are most probably global, even though the lichen-symbiotic participants may vary.

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The effect of extraterrestrial stressors (UVC- and γ-radiation) on isolated photobionts of the two astrobiological model lichens *Buellia frigida* and *Circinaria gyrosa*

JOACHIM MEESSEN[1], THERESA BACKHAUS[1], ROSA DE LA TORRE[2], MARINA RAGUSE[3], RYUICHI OKAYASU[4], RALF MÖLLER[3], SIEGLINDE OTT[1] AND THE HIMAC TEAM[4]

[1] *Institute of Botany, Heinrich-Heine-University Duesseldorf*
[2] *Departamento de Observación de la Tierra, INTA, Madrid*
[3] *Department of Radiation Biology, Institute of Aerospace Medicine, DLR, Cologne*
[4] *NIRS, Chiba*

Previous studies investigated the viability and photosynthetic activity of lichens after exposure to simulated and real space-parameters demonstrating high capacities of survival and recovery (de Vera *et al.* 2004a, 2004b, Onofri *et al.* 2012, Sánchez *et al.* 2012, 2014, Brandt *et al.* 2014). Consequently, the lichens *Buellia frigida* and *Circinaria gyrosa* are used as symbiotic models in on-going astrobiological research. In July 2014 they were launched to space as part of the BIOMEX experiment on EXPOSE-R2/Zvezda at the ISS where they are currently exposed to space and Mars conditions for 12-15 months.To determine their resistance in detail, we exposed metabolically active photobionts of both lichens to UVC alone and in combination with desiccating and freezing conditions in the laboratory. We also exposed them to γ-radiation of ≤112 kGy at the HIMAC-facility in Chiba, Japan. The dose- and recovery-dependent effects were examined by chlorophyll a fluorescence (quantum yield, non-photochemical quenching) as well as by DNA-degradation, total protein content and content of photosynthetic pigments. Depending on the UVC- and γ-radiation doses, the results indicate a strong impairment of photosynthetic activity and photo-protective mechanisms and revealed pronounced chlorophyll bleaching. Interestingly, combinations of UVC with stressors such as desiccation and subzero temperatures were found to attenuate UVC-damages putatively conferring an additive protective effect.In conclusion, the present study stresses the susceptibility of photobionts towards extreme extraterrestrial conditions as UVC- and γ-ray exposure. By comparison with previous studies on entire lichen thalli, the importance of anhydrobiosis and those protective mechanisms are stressed that are conferred by the symbiotic state (morphological features and secondary lichen compounds). The finding that the photobionts' reaction to one stressor attenuates the effect of another one – even a non-terrestrial stressor as UVC – may be one factor to explain the high resistance of lichens observed in previous astrobiological studies.

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Lichens as micro-ecosystems: novel approaches to efficiently reveal the hidden diversity of phycobionts in a single thallus

ARANTXA MOLINS[1], PATRICIA MOYA[1], FRANCISCO GARCÍA-BREIJO[2], JOSÉ REIG-ARMIÑANA[1], EVA BARRENO[1]

[1] *Instituto ICBIBE, Dpto. Botanica, Jardi Botanic, Fac. C. Biologicas, C/ Dr. Moliner 50. 46100-Burjassot, Valencia, Spain.*
[2] *Dpto. Ecosistemas Agroforestales, Universidad Politecnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain*

Scientists are reviewing Lichen characterization; these organisms are more complex than the marriage between a mycobiont and a phycobiont or cyanobiont because non-photoautotrophic lichenic bacteria seem to also be implied in the symbiogenesis of thalli. Additional complexity was reported inside a single lichen thallus by the intrathalline coexistence of different algal species and/or genera. Thus, lichen thalli should be considered as micro-ecosystems, taking into account the phycobionts as primary producers. Most of the studies on diversity and population structure within entire thalli were based only on the SANGER methodology that probably underestimated the potential microalgal heterogeneity. In 2010, our "Lichen Symbiogenesis Team" developed a simple and rapid method to isolate lichen phycobionts from fruticose, foliose and crustose lichens, which permits many pure, easily identifiable, axenic colonies of microalgae to be obtained in approximately 3 months. The aim of this study was to obtain a faster and more accurate methodology to analyse the whole of the potential diversity in the symbiotic primary producers.We have improved the procedure by shortening the full process to 45 days, following the identification by SANGER of pure axenic colonies. We studied the phycobiont relationships inside the thalli of *Flavoparmelia soredians* (Nyl.) Hale and *Circinaria hispida* (Mereschk.) A. Nordin, S. Savić & Tibell. Isolated microalgae of both lichen species have been characterized with molecular and ultrastructural techniques. We were able to detect in one single thallus of *C. hispida* the coexistence of at least four *Trebouxia* and two *Coccomyxa* taxa; nevertheless in *F. soredians* only one *Trebouxia* taxon was identified. Our results suggest that this method may be an appropriate protocol before studying symbiotic microalgae relationships inside individual lichen thalli in order to more efficiently reveal its hidden diversity.

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Rock-inhabiting fungi and their association with algae: where does symbiosis start?

LUCIA MUGGIA[1,4], LAURA SELBMANN[2], KERRY KNUDSEN[3], MARTIN GRUBE[4]

[1] *Department of life Science, University of Trieste, Via L. Giorgieri 10, Trieste, Italy*
[2] *Università degli Studi della Tuscia, Largo dell' Università, Viterbo, Italy*
[3] *Department of Ecology, Faculty of Environmental Sciences, Czech University of Life*
[4] *Karl-Franzens-Universität Graz, Institute of Plant Sciences. Schubertstraße 51, 8010 Graz, Austria*

Black fungi are ubiquitous colonizers of rock surfaces but the knowledge about their morphological and genetic diversity and any interaction with algae co-occurring on the rocks is still limited. Culture-dependent and molecular phylogenetic approaches have been used to describe new species and new genera from different extreme habitats of the world. Culture experiments have been further applied to test whether these fungi can associate with algae *in vitro*. The majority of rock-inhabiting fungi lack sexual reproductive structures and genera have been characterized on the base of anatomical characters of mycelia. We present a reappraisal of the phylogenetic relationships of rock-inhabiting fungi belonging to the *Dothideomyetes* from diverse environments and sharing diverse life styles. Our analyses of environmental samples and culture isolates reveal that the fertile genus *Lichenothelia* is paraphyletic and one lineage is closely related to the anamorphic genus *Saxomyces*. In addition we tested the capacity to form lichen-like relationships with algae in this *Lichenothelia-Saxomyces* complex using culture experiments. The experiments show various types of interactions with *Trebouxia* and *Coccomyxa* algae, which also sheds new light on the life-style flexibility of these rock-inhabiting fungi.

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Water relations in lichens: a case study with the tripartite *Peltigera britannica* and its isolated photobionts

FRANCESCO PETRUZZELLIS[1], FABIO CAUDURO[1], STEFANO BERTUZZI[1], TADEJA SAVI[1], TERESA CRAIGHERO[1], ALICE MONTAGNER[1], LUCIA MUGGIA[1], TOR TØNSBERG[1], MAURO TRETIACH[1], ANDREA NARDINI[1]

[1] *University of Trieste, Department of Life Science, via Giorgieri 10, Trieste, Italy*

In the past decades only few studies considered the water potential (Ψ) of lichens as a tracker of metabolic activity. Recent studies confirmed the correlation between Ψ and photosynthesis, but they also suggested the need of further analyses at the photobiont level, since some of the observed differences might actually be related to the type of photobiont, namely green algae vs. cyanobacteria. *Peltigera britannica* is a tripartite macrolichen hosting the green alga *Coccomyxa* sp. in the thallus and the cyanobacterium *Nostoc* sp. in the external cephalodia. Occasionally a large-lobed blue-green morphotype develops from the cephalodia and becomes independent. In this study the water relations parameters and chlorophyll fluorescence emission were compared among samples of the green and the blue-green morphotypes and the axenic cultures of the individual photobionts. Water potential isotherms were analyzed to calculate: π0 (osmotic potential at full turgor), Ψtlp (water potential at the turgor loss point), and ε (bulk modulus of elasticity). These traits provide information on the capability of maintaining cell turgor upon water loss, which is fundamental for lichens to extend the duration of photosynthesis and cell respiration. The aims are (i) to assess possible differences in the water relations parameters of the intact thallus lobes vs. the isolated photobionts, and (ii) to highlight the relationship between cell turgor and photosynthetic activity. Thallus lobes containing the green photobiont show a better capability in maintaining cell turgor as a result of lower values of π0 and Ψtlp. Ψtlp was also identified as a critical value after which the activity of the photosystems decreases significantly. This allows tracking photosynthesis using the cellular turgor state instead of the thallus relative water content, that is still widely used in the lichenological literature although it is less precise and definitely less appropriate in defining the water status of a cell.

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Challenging symbiont association patterns in lichens

LUCIA MUGGIA[1], PAVEL SKALOUD[2], JANA STEINOVA[2,3]

[1] *Department of Life Science, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy*
[2] *Department of Botany, Charles University in Prague, Benatska 2, 12801 Prague, Czech Republic*
[3] *Institute for Nanomaterials, Advanced Technologies and innovation*

Lichen symbioses are complex and coordinate associations between fungi (mycobionts) and algae (photobionts). The associated partners can propagate either independently or jointly. The independent propagation forces the mycobionts to find and reassociate with suitable photobionts. Alternatively, the joint dispersal of the symbionts avoids the problems of possible low partner availability in the environment and allows faster colonization of free habitats. Variable patterns of mycobiont-photobiont associations have been detected in lichens, but it is still unclear how this variation correlates with symbiont selectivity patterns, especially in lichens that are geographically widespread and form species-complexes or adopt different dispersal strategies. Here we present studies based on molecular and culture-dependent approaches of mycobiont and photobiont diversity in two groups of lichens, crustose and fruticose, that mirror the two categories. Crustose species of the *Tephromela atra* complex are geographically widespread, they present a continuum of morphological diversity, and their lineages show different degrees of specificity towards photobionts of the genus *Trebouxia*. Alternatively, fruticose species of red-apotheciated *Cladonia* associate with the photobiont genus *Asterochloris* and are characterized by different dispersal strategies. Monophyletic lineages of *Tephromela* and *Cladonia* species that jointly disperse their symbionts are characterized by high photobiont specificity; alternatively a broad range of *Trebouxia* and *Asterochloris* species are found in poorly resolved *Tephromela* lineages and in sexually reproducing *Cladonia*, respectively.

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How does *Cladonia portentosa* respond to nitrogen? Effect of form, dose, time of exposure, and PK addition on protein expression

SILVANA MUNZI[1], LUCY SHEPPARD[2], CRISTINA CRUZ[1], CRISTINA BRANQUINHO[1], LUCA BINI[3], LUIGI PARROTTA[3], GIAMPIERO CAI[3]

[1] *Center for Ecology, Evolution and Environmental Change, Universidade de Lisboa, Portugal*
[2] *Centre for Ecology & Hydrology, Edinburgh, UK*
[3] *Dipartimento di Scienze della Vita, Università degli Studi di Siena, Italy*

One of the first effects of reactive nitrogen excess on ecosystems is the shift in the lichen component with the disappearance of oligotrophic species and the increase of nitrophytic ones. Recent findings suggest that tolerant species exposed to high nitrogen availability are able to develop mechanisms to cope with increasing nitrogen in the environment.In this work, we performed proteomic analyses in thalli of *Cladonia portentosa* exposed to different forms and doses of nitrogen under controlled conditions in order to investigate how protein expression changes in relation to nitrogen availability. Moreover, samples exposed for 6 months were compared with samples exposed for 11 years to check possible adaption mechanisms in long-term treated lichens. Finally, we tested the effect of phosphorus and potassium addiction to investigate whether the alleviating effects of nitrogen toxicity symptoms observed for these elements operate through the modification of protein expression. Results showed a significant difference among the samples analyzed in protein composition related to six main functional categories: respiration, photosynthesis, protein synthesis, stress (chaperone/folding/oxidation), regulation and secretion. Different expression patterns were associated to different forms of nitrogen but only in few cases there was a linear relation with the dose. The PK supply influenced respiration and stress proteins while two proteins were expressed only in case of long term treatments. This contributes to the understanding of nitrogen tolerance in lichens and in particular of the adaptation mechanisms that are developed besides species-specific constitutive characteristics.

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Do saxicolous lichen communities represent ecological guilds assembled on locally adapted photobionts?

ONDŘEJ PEKSA[1], TEREZA ŘÍDKÁ[2], LUCIE VANČUROVÁ[2], ZUZANA VAIGLOVÁ[2], PAVEL ŠKALOUD[2]

[1] *The West Bohemian Museum in Pilsen*
[2] *Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, Praha 2, 128 01*

Lichen ecological guilds are defined as the communities of lichens growing in the same habitat, sharing the same pool of locally adapted photobionts (i.e. symbiotic algae or cyanobacteria). Although many lichen communities have been described based on the composition of lichen fungi, almost nothing is known about the composition of their photobionts. By the ITS rDNA genotyping, we have studied the diversity, sharing, and switching of photobionts within and among the three selected green-algal saxicolous lichen communities. These communities shared the climatic conditions, but differed by a substrate type. Specifically, we investigated the communities grown on i) siliceous rocks (*Parmelion conspersae*), ii) siliceous rocks with high heavy metal content (*Acarosporion sinopicae*), and iii) limestone rocks (*Aspicilion calcareae*). The results corroborate the hypothesis of the existence of a restricted photobiont pool associated with particular lichen communities. In addition, our research brings interesting findings about the ecology of photobionts and general mechanisms underlying the formation and functioning of lichen guilds.

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Environmental preferences of photobionts associating with epigeic *Cladonia* communities

TEREZA ŘÍDKÁ[1], ONDŘEJ PEKSA[2], PAVEL ŠKALOUD[1]

[1] *Charles University in Prague, Czech Republic*
[2] *The West Bohemian Museum in Pilsen, Kopeckého sady 2, 30 100 Plzeň, Czech Republic*

Little is known about the ecology of lichen photobionts so far. However, some recent studies observed that physiological responses to abiotic factors, such as temperature and light, may differ even between closely related photobionts. Thus, to ensure high fitness of the whole lichen system, the mycobiont should find the suitable photobiont genotype flourishing in local conditions. We studied the diversity of photobionts in terricolous lichen communities growing in habitats differing especially by the character of substrate (e.g. heaths on sandy soils, limestone steppes, schist and serpentinite rocks). By ITS rDNA genotyping, we found several different lineages of green alga *Asterochloris* associating with 42 studied *Cladonia* species. In general, the results indicated the intensive sharing of photobionts among fungal taxa with similar ecology, i.e. different environmental requirements of individual photobiont lineages. Substrate pH and climate seemed to be the most important factors. Our results clearly showed that particular *Cladonia* communities associate with different combinations of photobionts, forming specific lichen guilds – communities of lichens growing in the same habitat and sharing the same photobiont pool.

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Diversity, climate related distribution and species specificity of Antarctic lecideoid lichens

ULRIKE RUPRECHT[1], GEORG BRUNAUER[1], CHRISTIAN PRINTZEN[2]

[1] *University of Salzburg*
[2] *Senckenberg Forschungsinstitut und Naturmuseum Frankfurt*

The Antarctic continent with its most extreme climate conditions provides an environment where only the hardiest organisms can survive. The terrestrial vegetation communities of the ice-free areas of this continent (c. 2%) are mainly comprised of mosses, fungi and lichens. However, the mechanisms that connect climate and life's diversity in Antarctica are still poorly understood owing to limited taxon and climate data sampling in many areas of the continent. The current study investigates the role of climatic factors driving lichen diversity, distribution and species specificity in Antarctica using saxicolous lecideoid lichens as a model system. These lichens are perfectly adapted to the cold and severe habitats and abundant throughout the whole Antarctic continent.The mycobionts of lecideoid lichens in Antarctica are associated with several *Trebouxia* species as photobiont. The various mycobiont species show different patterns of distribution, genetic diversity and phylogenetic relationships to their photobionts. The molecular results so far, show also an evidence for the influence of climatic factors on the distribution and genetic diversity of the *Trebouxia* species. Most *Trebouxia* species are cosmopolitan, but also have preferences to special climate conditions. Recently, a newly identified species (T. sp. URa1) was found as to be a local endemic of the most extreme cold deserts. There is still the question, why is a lichen species such as *Lecidea cancriformis* abundant all over the continent and in contrast *Lecidella greenii* restricted to milder and humid areas? One major factor may be the choice of the photobiont since the molecular data show for *L. cancriformis* a low species specifity to its photobiont and hence the ability to choose different *Trebouxia* species, which are available all over the continent. *Lecidella greenii* is restricted to only one *Trebouxia* species which merely occurs to the milder habitats.

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Transcriptomics of desiccation tolerance in Antarctic lichen photobionts

ANDRES SADOWSKY[1], SIEGLINDE OTT[1]

[1] *Institute of Botany, Heinrich Heine University*

Antarctic terrestrial biota are characterized by low temperatures as well as moisture availability, high insolation, and short vegetation periods. Despite these harsh conditions, the lichen symbiosis survives and largely forms the dominant vegetation. The question arises which mechanisms enable lichenised associations to dominate Antarctic terrestrial habitats, and what is the molecular basis? Although desiccation is a common effect at natural lichen habitats, the recent knowledge about the genetic basis of desiccation tolerance in lichen photobionts is scarce. Transcriptomic studies are suitable to investigate and compare differential gene expression. The analysis of transcription in the lichen symbiosis under stress conditions is a new field of research and has not been applied to Antarctic lichen photobionts before. A comparative transcriptomic study of the desiccation tolerance of two isolated photobionts is presented. These green algae isolated from the Antarctic lichens *Umbilicaria decussata* and *Usnea lambii* have previously shown differential physiological responses to abiotic stressors such as light, cold and drought. Of the c. 10,000 transcribed genes detected in each photobiont, c. 10-15% experience differential expression upon desiccation. The cellular functions influenced include heat-shock proteins and parts of the antioxidative pathways, as well as photosynthesis, growth and cell proliferation. Especially, the up-regulation of heat shock proteins (predominantly of the Hsp70 or Hsp40/DNAJ type) in the *U. decussata* photobiont and their high constitutive expression in the *U. lambii* photobiont are conspicuous. These results indicate that protection of protein conformation may be a key factor in desiccation tolerance, while the strategy can be based on constitutive or stress-induced mechanisms. These innovative results of the molecular mechanisms of stress tolerance of photobionts reveal the potential of carbon acquisition under stress conditions. The results foster the knowledge on the role of photobionts in the lichen symbiosis and the holobiont's potential to resist severe environmental conditions.

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Diversity of mycobiont-photobiont associations correlate with reproductive strategies in *Cladonia* lichen species

JANA STEINOVA[1,3], LUCIA MUGGIA[2], PAVEL SKALOUD[3]

[1] *Institute for Nanomaterials, Advanced Technologies and innovation, Bendlova 1407/7, 46117 Liberec, Czech Republic*
[2] *Department of Life Science, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy*
[3] *Department of Botany, Charles University in Prague*

In lichen symbiosis, associated partners propagate either independently or by dispersal of joint propagules, such as isidia or soredia. Joint dispersal of symbionts avoids problems of possible low partner availability in the environment, and allows fast colonization of free habitats. On the other hand, independently dispersed bionts must always find the suitable partner and establish the lichen thallus de novo. According to several recent studies, dispersal strategies seem to influence the diversity of associated partners. We studied the diversity of four zeorin-containg, red-fruited *Cladonia* mycobionts species associated with *Asterochloris* photobionts. Two of them, *Cladonia deformis* and *C. pleurota*, are characterised by the production of soredia, whereas *C. coccifera* and *C. diversa* reproduce mostly sexually by ascospores. We sequenced two fungal (ITS rDNA and β-tubulin gene) and two algal genetic markers (ITS rDNA and intron-containing actin gene) of 84 *Cladonia* specimens sampled from different localities in Europe. Our results show clear patterns of low photobiont diversity in *Cladonia* species with joint dispersal strategy compared to species with predominant sexual reproduction. While we detected only two *Asterochloris* lineages associated with sorediate species, ten *Asterochloris* lineages have been found in esorediate species. Our results suggest that different dispersal strategies of symbiotic partners may strongly affect photobiont diversity, and possibly lead to a co-evolution of associated symbionts.

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Unexpected phycobiont diversity of *Stereocaulon* lichens

LUCIE VANČUROVÁ[1], ONDŘEJ PEKSA[2], PAVEL ŠKALOUD[1]

[1] *Charles University in Prague, Faculty of Science, Department of Botany, Benátská 2, 128 01 Prague 2, Czech Republic*
[2] *The West Bohemian Museum in Pilsen, Kopeckého sady 2, 301 00 Plzeň, Czech Republic*

Stereocaulon (Lecanorales, Ascomycota) is a genus of lichenized fungi with a broad ecology and an extensive geographical distribution. It ranks among the pioneer lichens growing in harsh conditions on newly formed substrates. Since the lichen ecology may be greatly influenced by the needs of their phycobionts, the major goal of this study was to describe the phycobiont diversity of *Stereocaulon* lichens, and to find a connection of this diversity and environmental conditions. The methods involved sequencing ITS rDNA, actin type I, rbcL and 18S rDNA from thalli of several *Stereocaulon* species collected in a broad range of localities and habitat types. Although a single algal genus, *Asterochloris* (Trebouxiophyceae), was associated with the most of sampled *Stereocaulon* thalli, three other Trebouxiophycean genera were also found to form lichen associations with the genus. First, the common free-living alga *Chloroidium* was recorded as a phycobiont in numerous thalli growing on volcanic or artificial substrates. This data confirms previous sporadic findings of *Chloroidium* phycobionts in thalli of some lichens. On the contrary, representative of genus Watanabea was found as a phycobiont for the first time. Finally, a novel, undescribed Trebouxiophycean lineage of lichen phycobionts was revealed in several *Stereocaulon* lichens. Our data demonstrates that the diversity of lichen phycobionts is still underestimated and poorly known. The unexpected diversity of *Stereocaulon* phycobionts is probably connected with extreme and diverse environmental conditions in which these lichens grow. In each habitat type, they can select the best adapted alga as a phycobiont. Consequently, this ability of cooperation with various free-living algae could be highly advantageous for pioneer colonization of newly formed substrates.

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The impact of multiple photobionts on lichen ecology and distribution – an ecophysiological approach

ANNA VOYTSEKHOVICH[1], LYDIA GUSTAVS[2], ULF KARSTEN[2]

[1]*M.G. Kholodny Institute of Botany of the NAS of Ukraine, Tereshchenkivska str. 2, 01001 Kyiv, Ukraine*
[2]*University of Rostock, Applied Ecology and Phycology, Albert-Einstein-Str. 3, 18059 Rostock, Germany*

The photobiont flexibility of 20 lichen species (35 specimens) of the genera *Micarea, Placynthiella, Trapeliopsis, Baeomyces, Bilimbia* and *Epigloea* collected from Germany, The Netherlands and Ukraine was examined. 40% of analysed lichen specimens were associated with more than one photobiont simultaneously. The majority of the isolated photobionts belong to the green alga classes Trebouxiophyceae and Chlorophyceae, widely distributed in terrestrial habitats in free-living state (e.g. *Chloroidium*, *Coccomyxa sensu lato* including the genera, *Elliptochloris, Gloeocystis* and *Radiococcus*). These algae may form macroscopic biofilms on different substrates like soil, sand, rocks, concrete, bark of trees, decaying wood, mosses, and even at lichen-forming and saprobe fungi (Hoffmann 1989, Mikhailyuk *et al.* 2011, Kulichová *et al.* 2014). The cultivation of the isolated lichen photobionts under various photon flux densities (PFDs) revealed different physiological and morphological adaptation levels. *Coccomyxa* s. l. species favor relatively high PFDs, while *Elliptochloris subsphaerica, Radiococcus* and *Chloroidium* seem to prefer shaded or semi-shaded growth conditions. Our results indicate that free-living terrestrial algae are the main source of photobionts for studied lichen genera. The ecophysiology of these algae plays an important role in lichen ecology and distribution. A certain liability in photobiont choice and presence of multiple photobionts in thallus helps lichen forming fungi to colonize different biotops and substrates.

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Optionally lichenized fungi of *Hyphodontia* (Agaricomycetes, Schizoporaceae)

ANNA VOYTSEKHOVICH[1], TATIANA MIKHAILYUK[1], YURI AKIMOV[1], ALEXANDER ORDYNETS[2], LYDIA GUSTAVS[3], ULF KARSTEN[3]

[1] *M.G. Kholodny Institute of Botany, Tereshchenkivska str. 2, 01001 Kyiv, Ukraine*
[2] *Universität Kassel, Heinrich-Plett-Str. 40, 34132 Kassel, Germany*
[3] *University of Rostock, Albert-Einstein-Str. 3, 18059 Rostock, Germany*

Previously the fungi of *Hyphodontia J.* Erikss. genus were known only as a saprotrophs (Langer 1994). We found that at least 3 species - *Hyphodontia breviseta, H. crustosa* and *H. pallidula* occure as saprobe and lichen. Both lichenized and saprotrophic forms grow in the same habitats (on rotten wood and bark of different trees in humid conditions). Optionally lichenized specimens contained two distinct photobionts simultaneously. Algal species were isolated in culture. According to the molecular phylogeny of internal transcribed spacer (ITS) rDNA several distinct species related to genera *Coccomyxa* and *Elliptochloris* were identified. The results of both light and electron microscopy revealed two types of contact between fungal hyphae and algal cells. The first type is wall-to-wall contact when fungal hyphae tightly entwine photobiont cell, but do not enter into it. Contacts of this type are characteristic for basidial lichens, as well as representatives of *Peltigera*ceae and Lobariaceae that form associations with *Coccomyxa*, *Myrmecia* and *Dictyochloropsis* (Oberwinkler 1984, Honegger 1984, Brunner 1985). Another type of contact is intraparietal, when hypha (haustorium) lyses the cell wall of alga and penetrates the algal cell. Such contacts are characteristic for lichen-forming fungi that are associates with some green algae (e.g. *Pseudochlorella* and *Elliptochloris*) – *Lecidea* and *Micarea* (Brunner 1985), and cyanoprokaryota (*Scytonema*) – basidiolichen *Dictyonema* (Oberwinkler 1984).

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Diversity of algal component in saxicolous lichen communities of Karadag Nature Reserve (Crimea)

ANNA VOYTSEKHOVICH[1], ANDREAS BECK[2]

[1]*M.G. Kholodny Institute of Botany, Kyiv, Ukraine*
[2]*Botanische Saatssammlung München, Germany*

While the Karadag Nature Reserve is one of the richest and most unique places in the world for lichen diversity, the algal component of these lichens is scarcely known. In total we identified 26 taxa of photobionts from 10 genera (*Asterochloris*, *Chloroidium, Dilabifilum, Diplosphaera, Gloeocapsa, Myrmecia, Nostoc, Printzina, Stichococcus, Trebouxia*). As most of the studied lichens (more than 80 % of lichen species) were associated with *Trebouxia*, for which morphology-based identification is often difficult and not sufficient, we additionally used molecular methods. According to the molecular phylogeny of internal transcribed spacer (ITS) rDNA, *Trebouxia* species were related to three main clades: “Arboricola” (88.9% of the investigated *Trebouxia*-containing lichens), “Impressa” (4.4%) and “Simplex” (6.7%). It was observed that the physicochemical properties of the substrate on which the lichen grew did not have a strong impact on the photobiont composition. In contrast, it was shown that the photobiont composition was influenced by the selectivity of the mycobiont. Lichens were characterized by different levels of selectivity, although the lichens with high and middle selectivity levels were dominant. *Aspicilia cinerea, Candelariella medians, C. vitellina, Circinaria contorta, Diploschistes diacapsis, Lepraria membranacea, Ramalina calicaris, R. capitata, Rhizocarpon geographicum* and *Staurothele* sp. contained two photobionts simultaneously.

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Session Chairs: [1]Irene L.G. Newton, [2] Luis Teixeira
Chair affiliations: [1]Indiana University, USA
[2]Instituto Gulbenkian de Ciência, Portugal

Molecular and cell biology of *Wolbachia*-host interaction

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Mutualism breakdown by amplification of *Wolbachia* genes

EWA CHROSTEK, LUIS TEIXEIRA

Instituto Gulbenkian de Ciência, Oeiras, Portugal

Microbes form complex and intimate relationships with their animal hosts. *Wolbachia* is a vertically transmitted bacterium that lives within the cells of numerous arthropod species. *Wolbachia* is a famous manipulator of insect reproduction: to favor its own spread it can induce male killing, parthenogenesis or cytoplasmic incompatibility. *Wolbachia* can also protect various insects from pathogens, which makes it a potential tool for the control of vector-borne diseases. Field trials are already being conducted, involving releases of mosquitoes with *Wolbachia* in the wild. Yet, how *Wolbachia* manipulates its hosts remains largely unknown. To gain an insight into *Wolbachia*-host interaction we have recently compared different closely related *Wolbachia* variants in *Drosophila melanogaster*. The strength of antiviral protection, symbiont titers and host longevity allowed us to divide the variants into distinct phenotypic groups matching phylogeny based on complete genome sequences. Moreover, analysis of an over-replicating and life-shortening *Wolbachia* variant led us to discover the cause of its virulence at the genome level. Using experimental evolution we show that virulence of the symbiont can change rapidly and that regulation of its titers can be broken with a single genetic change in *Wolbachia*. Therefore, our results provide the first link between genotype and phenotype in this unculturable endosymbiont.

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Dynamics of *Wolbachia pipientis* gene expression across the *Drosophila melanogaster* life cycle

FLORENCE GUTZWILLER[1] , CATARINA R. CARMO[2] , DANNY E. MILLER[3,4] , DANNY W. RICE[5] , IRENE L. NEWTON[5], LUIS TEIXIERA[2] , CASEY M. BERGMAN[1]

[1] *University of Manchester, Manchester, UK*
[2] *Instituto Gulbenkian de Cincia, Oeiras, Portugal*
[3] *Stowers Institute for Medical Research, Kansas, USA*
[4] *University of Kansas Medical Center, Kansas, USA*
[5] *Indiana University, Indiana, USA*

Symbiotic interactions between microbes and their multicellular hosts have manifold impacts on organismal and cellular biology. The α-proteobacteria *Wolbachia pipientis*, a widespread endosymbiont, causes diverse phenotypic effects on its hosts ranging from reproductive manipulation to viral protection. To understand how *Wolbachia* interacts with its host, we analyzed genome-wide patterns of *Wolbachia* gene expression across the life cycle of *Drosophila melanogaster* using public RNA-seq datasets from strains with previously-undetected *Wolbachia* infections. In a strain carrying a wMel genotype, we find that the majority of *Wolbachia* genes are expressed at detectable levels across all embryonic, larval, pupal and adult stages sampled, and that only 6.7% of *Wolbachia* genes exhibit reproducible, stage-specific differences at the level of the whole organism. The majority of differentially-expressed genes in this strain are up-regulated after embryogenesis, including many genes that have functions in the bacterial cell membrane that suggest direct interactions with the host, as well as operons of uncharacterised genes that are expressed highly in adult males and may play a role in cytoplasmic incompatibility. Analysis of *Wolbachia* expression in an independent line of *D. melanogaster* carrying a wMelCS genotype confirms that the majority of *Wolbachia* are expressed and that few *Wolbachia* genes differ across *D. melanogaster* life-cycle stages, but also reveals the identity of differentially-expressed genes and their developmental dynamics is context dependent. Our results indicate that large changes in *Wolbachia* gene expression across the *Drosophila* life-cycle are relatively rare at the whole-organism scale, consistent with the limited regulatory capacity encoded by the *Wolbachia* genome and the stable environment provided by endosymbiosis. Our work also shows that mining public gene expression data in *Drosophila melanogaster* provides a rich set of resources to probe the functional basis of the *Wolbachia-Drosophila* symbiosis and to annotate the transcriptional landscape of the *Wolbachia* genome.

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Session: Molecular and cell biology of *Wolbachia*-host interaction
Presentation type: Oral
Name, Contact: Kaur Rupinder, rimzz.mht@gmail.com

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Symbiosis and Immunity: Spatio-temporal infection dynamics of *Wolbachia* and RNA viruses in *Drosophila suzukii* and related species

RUPINDER KAUR[1], [2], JULIEN MARTINEZ[3], FRANK JIGGINS[3], OMAR ROTA-STABELLI[2], WOLFGANG J. MILLER[1]

[1] *Lab Genome Dynamics, Department of Cell and Developmental Biology, Medical University of Vienna, Austria.*
[2] *Chemical Ecology Lab, Dept of Sustainable Agro-Ecosystems and Bioresources, FEM, Italy.*
[3] *Dept of Genetics, University of Cambridge, Cambridge, UK*

Bacteria, viruses and the host organism altogether form a tri-partite symbiotic association with significant impacts on host fitness and fecundity. In insects, endosymbiotic bacteria '*Wolbachia*' can manipulate host reproductive biology to increase their own transmission. Recently, they have been shown to protect insects from a range of RNA viruses, offering them a fitness boost in exchange for a place to live. Newly emerged research model *Drosophila suzukii* is an invasive fruit pest in America and Europe, harboring a new strain of *Wolbachia* –wSuz. This strain is closely related to wRi strain of *D. simulans* (Siozios *et al,* 2013). Till date, no significant levels of *Wolbachia*-directed CI could be found in *D. suzukii*, despite the fact that this endosymbiont has an ancestral symbiotic relationship; with stable polymorphism in the field populations of both continents. This suggests balancing host-symbiont selection, whose nature and extent is not known yet. Here we report on *Wolbachia* capability to provide protection against RNA viruses i.e. Flock House virus (FHV) and *Drosophila* C Virus (DCV) in *D. suzukii*, as well as in their sister species *D. subpulchrella*. Our finding of strong protection against viral pathogens is in agreement with early hypothesis that *Wolbachia* prevalence in natural populations is balanced by induced resistance against native RNA viruses. Comparing our results of long-term association system of wSuz-*D. suzukii* to that of recent infection system of wRi-*D. simulans*, we found that in *D. suzukii* group, antiviral protection ability does not directly correlate with global *Wolbachia* density in whole flies per se, but with defined host tissue tropism. Currently we are investigating the relationship between *Wolbachia*- and virus-tropism after proliferation among different host tissues using qRT-PCR, Fluorescent in-situ hybridization and Immunofluorescence techniques. The hypothesized co-localization of *Wolbachia* and viruses will give new insights into their co-evolution in the respective host organism.

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Wolbachia wCle discovery in the bedbug genome

SERGIOS-ORESTIS KOLOKOTRONIS[1], JEFFREY ROSENFELD[2], DARRYL REEVES[3], PAUL J PLANET[4], ALEX KENTSIS[5], LOUIS SORKIN[6], MARK E. SIDDALL[6], ROB DESALLE[6], GEORGE AMATO[6], CHRISTOPHER E. MASON[3]

[1] *Fordham University*
[2] *Rutgers University*
[3] *Weill Cornell Medical College*
[4] *Columbia University*
[5] *Memorial Sloan Kettering Cancer Center*
[6] *American Museum of Natural History*

The common bedbug (*Cimex lectularius*) has been a persistent pest of humans for thousands of years, yet the genetic basis of the bedbug's basic biology and adaptation to dense human environments is largely unknown. For eukaryotic organisms, the microbiome is an important part of overall metabolism and physiology. We produced the assembly, annotation, and phylogenetic mapping of the 697.9Mb *Cimex lectularius* genome (N50=820kb). We examined the bedbug microbiome using reciprocal TBLASTX and MUMmer searches of the bedbug genes against all bacterial genomes on GenBank. Of the 1,509 genes from 436 different bacterial species, the top matches were *Wolbachia* (114 genes), followed by *Clostridium* (25 genes). An RNAseq time-course across all five developmental stages and male and female adults generated 36,985 gene models. Most differentially expressed genes during the bedbug life cycle occurred after feeding on human blood and included genes from the *Wolbachia* endosymbiont (wCle). These results show a simultaneous a coordinated host-commensal response to hematophagy and provide an annotated set of genes linked to the wCle function as an endosymbiont. The most polymorphic wCle genes belong to pathways that may have different fitness significance for intracellular symbionts, e.g. the gene ddl coding for D-alanine–D-alanine ligase that is involved in peptidoglycan biosynthesis and is required for cell wall synthesis. Structural homology modeling of DDL (PDB ID: 1IOV, 53% similarity to *E. coli*) showed the most notable structural changes to be localized in the ATP-binding pocket with strand 95-98 shifted to allow more flexibility for ATP binding. While the two bedbug wCle ddl sequences showed no signs of diversifying selection within the broader *Wolbachia* context, they harbored 8 aa differences. As a subset of a eukaryotic genome characterization project, our study shows that intraspecific variation does exist in this obligate endosymbiont, potentially conferring fitness variation on the bedbug host.

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Comparative genomics of *Wolbachia*: Evolution of genome content in a reproductive parasite

AMELIA LINDSEY, RICHARD STOUTHAMER

Department of Entomology, University of California, Riverside, CA

Wolbachia is a bacterial endosymbiont of arthropods and nematodes, well known for modifying reproduction of its host. Within *Wolbachia*, there is a series of distinct, monophyletic supergroups, host-switching appears to be commonplace amongst arthropods, and there is little conservation of the induced reproductive phenotypes. We present a draft genome for wTpre infecting the parasitic wasp, Trichogramma pretiosum, representing the most complete genome of a parthenogenesis-inducing strain to date. The wTpre genome and seventeen other previously published *Wolbachia* genomes from 5 supergroups were used in comparative analyses after annotation and identification of clusters of orthologous genes. Across all 18 strains, the core genome is composed of 193 genes. We explored changes in genome content across supergroups, and find a significant increase in the number of ankyrin repeat domain genes in the B-supergroup of *Wolbachia*, which contains the newly sequenced wTpre strain. *Wolbachia* genomes varied most in the number of genes in the following three role categories: mobile and extrachromosomal element functions, transport and binding proteins, and cellular processes. A low number of phage genes were discovered in the wTpre and nematode-infecting strains – these *Wolbachia* strains were previously thought not to have any associated phages. The wClec bedbug-infecting strain from the F-supergroup contains a high number of phage genes, despite its phylogenetic position nested within the low-phage containing nematode strains. Changes in genome content are likely associated with host-adaptation, and may provide insight into the requirements for establishing and maintaining such a unique symbiotic relationship.

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Unorthodox transmission modes of endosymbionts in hybrids and the symbiotic origin of speciation

WOLFGANG J MILLER, DANIELA I SCHNEIDER

Medical University of Vienna

Although not a new idea, recent studies suggest that differences in the composition of symbiotic microbes between hosts can lead to reproductive isolation, and as a consequence also to speciation. Despite the fact that microbes are universally present in eukaryotes, they are rarely considered as a driving force of speciation, and chances are their contribution to speciation is overlooked. Symbiotic bacteria of the genus *Wolbachia* are known to affect their hosts' reproduction in adaptive manners to improve the propagation of the maternally transmitted endosymbiont throughout populations. These reproductive alterations that can result in postmating isolation via cytoplasmic incompatibilities, have recently been shown to foster also premating isolation in some host-symbiont associations such as the *Drosophila paulistorum* species complex, giving even more reason to assume that *Wolbachia* can play a significant role in host speciation.Here we will present most recent data on the involvement of *Wolbachia* in host speciation using two different insect systems, i.e., Neotropical *Drosophila* and African tsetse flies, both presently under incipient speciation in nature, carrying closely related but incompatible *Wolbachia* strains. We will also show that naturally incompatible and sterile hybrids of both systems can be rescued by means of even mild paternal *Wolbachia*-knockdown before forced mating, giving rise to fertile progeny and stable hybrid lines. Surprisingly such rescued hybrid lines show complete sexual isolation to parental lines as well as unambiguous signatures of paternal inheritance of both their cytoplasmic endosymbionts, i.e., of mitochondria plus *Wolbachia*.

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Wolbachia utilize actin for maintenance and transmission within *Drosophila melanogaster*

IRENE LG NEWTON, OLEKSANDER SAVYTSKYY, KATHY B SHEEHAN

Department of Biology, Indiana University

Wolbachia pipientis is a maternally transmitted bacterium that targets and replicates within the developing oocytes (in the germarium) of *Drosophila melanogaster*. Although *Wolbachia* are primarily maternally transmitted, they can achieve this localization even after injection into the fly abdomen, suggesting they are capable of transiting through multiple cell and tissue types in order to migrate to their final niche. Here, we identify host actin as important for the maintenance and transmission of *Wolbachia* in *Drosophila melanogaster*. We present our discovery that a *Wolbachia* infection is sensitive to the regulation of actin in the host; mutations in the host actin regulatory proteins profilin and villin, which alter stability and abundance of actin in the germarium, also alter *Wolbachia* titer and transmission fidelity. We provide evidence through crosses, molecular analyses (PCR, Western blots), RNAi knockdowns, quantification of actin, as well as cytological examination of developing oocytes. We characterize three mutant alleles in *Drosophila*, in addition to two deficiency stocks, each of which result in reduced titer and a *Wolbachia*-clearing phenotype. We therefor conclude that *Wolbachia* require actin for maintenance and faithful maternal transmission in *Drosophila melanogaster*. We end by describing the use of these mutant backgrounds to select for mutant *Wolbachia* and their phenotypic characterizations.

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Wolbachia evolution in mutant *Drosophila* hosts

KATHY B SHEEHAN, MARYANN MARTIN, IRENE LG NEWTON

Department of Biology, Indiana University

Wolbachia pipientis is the most common bacterial endosymbiont of insects, infecting 40-60% of species. The bacteria are maternally transmitted via the germline of the host, but little is known about the mechanisms of host-symbiont interactions due to the difficulty of studying this non-culturable, intracellular parasite for which there is no genetic system. Here we present a method of generating *Wolbachia* mutants in the model organism, *Drosophila melanogaster*. By passaging *Wolbachia* through restrictive, mutant fly lines, we were able to bottleneck the bacteria and enrich for or induce genomic changes in only a few host generations. This method revealed loci potentially involved in adaptation of the microbe to its host ecosystem and showed that an intracellular symbiont can adapt rapidly to a mutant host context. To follow up on this work, we have passaged over 40 lineages of *Wolbachia* in a restrictive *Drosophila* background over ten generations. This experimental evolution project has identified both phenotypic and genomic changes in *Wolbachia* and has the potential to reveal much about host-microbe interaction.

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The fellowship of the ring: Is heme biosynthesis an influencing factor in *Wolbachia-Filarial* nematode endosymbiosis?

ASHLEY N LUCK, JEREMY M FOSTER, BARTON E SLATKO

New England Biolabs

Genomic sequencing has revealed that many human filarial nematodes, such *Wuchereria bancrofti, Brugia malayi* and *B. timori*, (causative agents of lymphatic filariasis (LF)) and *Onchocerca volvulus* (causative agent of onchocerciasis (river blindness)) contain the obligate endosymbiont, *Wolbachia*. Laboratory and human trials show that depletion of *Wolbachia* by antibiotics (e.g. doxycycline), can lead to blocking of embryogenesis, elimination of microfilariae (mf) output and adult worm killing. While the factors which are key to this obligate inter-relationship are not fully understood, genome sequencing identified a number of critical processes implicated in the host-endosymbiont interaction, one of which was heme biosynthesis. By serving as a co-factor in a number of enzymatic and biochemical processes, heme and heme regulation is essential to organism survival. Although *B. malayi* contains a functional ferrochelatase gene (the final step in the heme biosynthetic pathway and a product of lateral gene transfer), as with other nematodes they are incapable of synthesizing heme. However, the *Wolbachia* genome contains a complete and functional heme biosynthesis pathway, suggesting that *Wolbachia* may supply the host nematode with heme. Exploiting “NextGen” transcriptome and DNA sequencing, we have investigated differential expression patterns of the host and symbiont responses to heme in filarial nematodes. Numerous heme response genes (hrgs) from both organisms are differentially expressed in the presence of heme. Several show homology hrgs found in the free living nematode, *C. elegans*, that does not contain *Wolbachia*. RNAi and inhibitor studies in *B. malayi* further suggest heme homeostasis regulation may be important in the symbiotic relationship between the two organisms and thus might be a target for filariasis control.

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Session Chairs: Patricia Stock
Chair affiliations: University of Arizona, USA

Nematode-Bacteria symbioses

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Genomic signatures of coevolution in living and fossil *Wolbachia*

MARK BLAXTER

University of Edinburgh, Edinburgh, United Kingdom

Wolbachia is known as a parasitic endosymbiont of many terrestrial arthropods, where it induces a variety of reproductive phenotypes, the effects of which are to promote the reproduction of *Wolbachia*-transmitting females. *Wolbachia* also infect filarial nematodes, where the interaction has the features of mutualism. We are investigating the phylogenetic history of *Wolbachia* association with nematodes, and have identified fossil genomic evidence of *Wolbachia* in the genome of *Dictyocaulus viviparus*, a lung-parasitic strongylid nematode only distantly related to the filarial nematodes. The genomic fossils, ancient non-functional lateral gene transfers of *Wolbachia* DNA to the nucleus, reveal that the infecting *Wolbachia* was a member of supergroup F, a clade of *Wolbachia* known to currently infect both nematodes and arthropods. These findings suggest that (a) *Wolbachia* infection may have been (or still be) widespread in nematodes and (b) mutualism arose twice in filarial nematode *Wolbachia* and that supergroup F-like *Wolbachia* are likely ancestors of the supergroup C filarial *Wolbachia*.

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Presentation type: Keynote
Name, Contact: Bulgheresi Silvia, silvia.bulgheresi@univie.ac.at

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Dressed to cooperate: understanding symbiont spatial order on the host surface

NIKOLAUS LEISCH[1], NIKA PENDE[1], PHILIPP WEBER[1], TANNEKE DEN BLAAUWEN[2], SILVIA BULGHERESI[2]

[1] *Department of Bacterial Cell Biology, Swammerdam Institute of Life Sciences, Faculty of Science*
[2] *University of Amsterdam, Science Park 904, 1098 XH Amsterdam, the Netherlands*

Cell biological studies of bacterial symbionts are still very sparse. We studied four Gammaproteobacteria that thrive attached to the surface of their four respective nematode hosts in specific, inheritable spatial arrangements (e.g. bacterial “epithelia”, furs or knitting patterns). We showed that all four bacteria reproduce abnormally: the rod-shaped *Laxus oneistus* and *Robbea hypermnestra* symbionts grow in width, set their constricting rings longitudinally and give rise to epithelium-like layers. Symmetric transverse fission of up to 120 μm-long *E. dianeae* symbionts makes them the longest bacteria in which binary division has been observed, and give rise to fur-like bacterial coats. Finally, *Eubostrichus fertilis* symbiont cells divide at virtually every length between 4 and 45 μm. This results in a 10-fold length variation within the same cell population and in its knitting pattern-like arrangement. Although the observed extraordinary reproductive modes clearly mediate spatial order, what is the function of the latter? We are using a wide palette of classic and state-of-the-art microscopic techniques to address the ecological significance of the ectosymbionts’ spatial order.

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Ultrastructural, molecular and phylogenetic characterization of nematodes in the *Cecropia-Azteca* symbiosis, with emphasis on *Sclerorhabditis* sp. (nematoda: rhabditidae)

JESSICA MORERA, ADRIAN PINTO-TOMÁS

[1] *Universidad de Costa Rica, De la fundación Canadá, 50 metros sur, 50 metros oeste, Condominio Prados del Este casa D6 Montes de Oca San José, 157642 Costa Rica*

Recently ant-plant relationships such as the *Azteca-Cecropia* mutualism have been shown to include microbial associates. In this relationship, the *Cecropia* tree (Urticaceae) provides specialized structures for ant feeding and nesting, while *Azteca* (Dolichoderinae) provides protection against herbivores. The recent discovery of a novel nematode species, *Sclerorhabditis neotropicalis* (Rhabditidae), associated with this system prompted us to hypothesize that microscopic nematodes are consistently associated the *Azteca-Cecropia* system, being mobilized and transferred among trees by the ants. To start evaluating this hypothesis, *Cecropia-Azteca* samples were collected in 3 different regions within Costa Rica (Carara National Park, La Selva Biological Station and Metropolitan San Jose). The presence of nematodes was assessed and analyzed using light and electronic microscopy and phylogenetic analysis to identify and characterize isolated nematodes. We found associated nematodes in 100% of the analyzed trees (n=34), and our electron microscopy analyses allowed us to distinguish at least 5 different nematode species. The most commonly detected species belonged to the families Diploscapteridae and Rhabditidae. *Sclerorhabditidis* sp. was present in 94% of the sampled trees. To assess whether ants could transport the nematodes, we observed 170 *Azteca* worker ants under the microscope and recorded an average of 2 nematodes attached to the body of each insect. Given the prevalence of *Sclerorhabditidis* sp. in this system, we further characterized its ultrastructure by describing key anatomical features such as oral cavity, cuticle, vulva and tail employing electron microscopy, and sequenced 3 genes (Cox-I, 12S and 28S rRNA) to confirm their taxonomy and perform phylogenetic analyses. Our results suggest that *Sclerorhabditis* sp. isolates form a well-supported clade of bacteria-feeding nematodes associated with the *Cecropia-Azteca* symbiosis. Still, more studies are needed to clarify the type of relationship between *Cecropia-Azteca* and these nematodes. Further investigations should assess the possible benefits or harms that nematodes provide to this system.

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Electron microscopy, prokaryotic endosymbionts and changing concepts of the paradigms of parasitic associations

WIESLAW J KOZEK

Department of Microbiology, Medical Sciences Campus, University of Puerto Rico, San Juan, Puerto Rico

The broad spectrum of symbiosis includes parasitism: an association of two organisms, one of which (the parasite) lives in, or on, another organism (the host) - to the detriment of the host. Our studies to elucidate the ultrastructure of filariae – parasitic nematodes of man and animals - have revealed that these nematodes are themselves hosts of bacterial endosymbionts (*Wolbachia*). These Gram-negative bacteria are transovarially transmitted during the life cycle of the filarial, show tropism for the hypodermal tissue in all stages in the life cycle of filariae and for the germinal tissue of the females and appear to be essential for normal oogenesis and embryogenesis. Since *Wolbachia* have also been implicated in the pathogenesis of filarial infections, the host-parasite paradigm required modification to include this third component. Subsequent studies have shown that, in addition to *Wolbachia*, the filaria *Onchocerca volvulus* also harbors virus-like particles in the metabolic portion of its muscle cells, thus adding the fourth element to the paradigm. These findings have shown that some nematodes are susceptible to invasion by several types of prokaryotic organisms, rendering the host-parasite relationship that exists during filarial infections far more complicated than has been hitherto realized. Furthermore, they indicate the need to modify the classical paradigm of filarial infections to include the consideration of the following series of interactions: vertebrate host-filaria; filaria-*Wolbachia*; vertebrate host-*Wolbachia*; the virus-like agent-filaria; virus-like agent-vertebrate host; and virus-like agent-*Wolbachia*. The nature of the these associations requires further elucidation, especially the contribution that the organisms harbored by filariae contribute to the pathological manifestations of infection in the vertebrate host.

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Characterization of immune response genes in the parasitic nematode *Brugia malayi*

SILVIA LIBRO, JEREMY FOSTER, BARTON SLATKO

NEW ENGLAND BIOLABS, USA

The filarial nematode *Brugia malayi* is one of the causative agents of lymphatic filariasis, a neglected tropical that affects 120 million people worldwide. Due to the limited effectiveness of the drugs available and the absence of a vaccine, extensive efforts have been made in order to understand the basic biology of *B. malayi* and its symbiotic association with *Wolbachia* endobacteria. At present, little is known about the mechanisms underlying *Brugia*`s immune system. This overlooked area of research is of high interest, as and it can help to advance our understanding on the interaction between *Brugia* and *Wolbachia*. It has been suggested that in order to reside within the nematode`s tissue, *Wolbachia* may evade the host immune system. Therefore, understanding what mechanisms are involved in *Brugia* immunity can help in identifying targets for the development of new drugs and vaccines. In particular, small regulatory RNAs are potentially good candidate targets, as they are likely to be specific, decreasing the chances of off-target interactions. Several studies highlighted the key role of small regulatory RNAs during anti-viral immunity in *Caenorhabditis elegans* and suggest that *Brugia*, that possesses the genes encoding several components of the RNAi machinery, may use similar mechanisms to avoid parasites. In order to characterize the main genetic pathways involved in *B. malayi* immunity, we exposed adult male worms to four different immune elicitors: Gram + and Gram- bacterial lysates (*Escherichia coli* and *Bacillus sp*), dsRNA and dsDNA. We performed transcriptome and small RNA sequencing of worms exposed to each immune elicitors for two different exposure times (24 hours and 3 days). Differential gene expression analysis of untreated and immune challenged worms were used to characterize gene expression patterns associated to each type of immune insult and selected candidate immune genes were further validated using quantitative RT-PCR.

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Role of T6SS locus in *Xenorhabdus bovienii* (gamma-proteobacteria), the bacterial symbionts of *Steinernema* nematodes

JOHN G. MCMULLEN II[1], S. PATRICIA STOCK[2]

[1] *School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ, USA*
[2] *Department of Entomology, University of Arizona, Tucson, AZ, USA*

The tripartite *Steinernema* nematode – *Xenorhabdus bacteria* – insect host symbiotic interaction is a suitable model to understand mutualism and pathogenicity in host-microbe associations. The fitness of both *Steinernema* and *Xenorhabdus* are heavily dependent on the ability of the later to kill the insect host. It has been well established that a single *Steinernema* sp. harbors a specific *Xenorhabdus* sp. However, a single *Xenorhabdus* sp. may associate with more than one nematode species. Of all *Xenorhabdus* spp., *Xenorhabdus bovienii* has the widest known nematode host range by colonizing at least nine nematode hosts. In this study, we assessed the virulence of nine *X. bovienii* strains considering bioassays with different insect hosts coupled with a genomic approach. Bioassays indicated these strains vary in virulence to different insect hosts at the different lethal concentrations tested. From these results, we classified five strains as virulent and four strains as attenuated. Comparative genomic analysis correlated with the characterized insect mortality phenotypes depicted a type VI secretion system (T6SS) that was only found in all of the virulent strains. This locus was found to be similar to a T6SS in *Serratia marcescens*, which apparently plays a role in outcompeting other bacterial species. In this respect, we performed an *in vitro* intra-bacterial competition assay to determine the role of this T6SS in our model. Our assays between virulent and attenuated strains demonstrated that the virulent strains could outcompete and reduce attenuated strain populations. We predict this T6SS plays an important role in allowing *X. bovienii* to have colonized multiple *Steinernema* nematode hosts.

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Bacteria associated with *Bursaphelenchus xylophilus*: a role in Pine Wilt Disease or a positive role for the plant?

PAULA V MORAIS[1,2], DIOGO PROENÇA[2] AND ROMEU FRANCISCO[2]

[1] *Department of Life Sciences, University of Coimbra*
[2] *CEMUC, University of Coimbra*

Bacteria have been suggested to play a role on Pine Wilt Disease (PWD) since they have been isolated associated with the nematode. Therefore, isolates of *B. xylophilus* from across the globe have been studied in order to understand whether the bacteria isolated in association with nematodes can produce toxins that could play an important role in the development of the Pine Wood Disease (PWD). On a different perspective, the microbial community associated to PWN was assessed in nematodes from different affected areas in Portugal and from USA, geographical area where the disease comes from. The objective was to evaluate the diversity of the nematode-associated bacteria and evaluate their potential role by determining their *in vitro* nematicidal activity and their plant growth promoting potential. All isolates were screened for their ability to produce siderophores, lipases and proteases and 21 strains showed capacity to produce extracellular products with nematicidal activity against *B. xylophilus*. The most efficient nematicidal strain, *Serratia* sp. A88copa13, produced proteases in the supernatant with different nematicidal activity against different species of *Bursaphelenchus*. A second strain, *Serratia plymuthica*-like M24T3, showed potential to be a plant growth promoting bacterium due to its ability to produce IAA and siderophores, as well as to solubilize phosphate and zinc oxide. Additionally, it has an acdS gene encoding for 1-aminocyclopropane-1-carboxylate deaminase. When introduced into *Arabidopsis thaliana* tissues, exerted effects on plant development, decreasing plant root elongation but increasing the number of lateral roots.In conclusion, the work developed until now revealed the potential of some strains isolated in association with *B. xylophilus*, as antagonists against the phytopathogenetic nematodes as well as with direct and indirect plant growth-promoting properties. The bacteria's characteristics support a role in the protection of pine trees against the disease but further work is needed to demonstrate a direct interaction.

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Crude extracts of the entomopathogenic bacterium, *Photorhabdus l. sonorensis* (enterobacteriaceae) and their effect on two plant parasitic nematodes: *Meloidogyne incognita* and *Anguina pacificae* (nematoda: *Tylenchina*)

ROUSEL A OROZCO[1], S PATRICIA STOCK[1]

[1] *Department of Entomology, University of Arizona*

Photorhabdus spp. are Gram negative bacteria that are mutualistically associated with *Heterorhabditis* nematodes. *Photorhabdus* use *Heterorhabditis* nematodes as vectors which help them access a wide range of soil inhabiting insect species. Once the bacteria are delivered by the nematodes in the insect host, they release potent toxins and secondary metabolites that kill the insect and also provide suitable environment for the nematode to grow and multiply. Therefore, *Photorhabdus* has dual role in its lifecycle: mutualist of the nematodes and pathogen to the insects. Secondary metabolites, produce by these bacteria are known for their wide biological activity spanning from insecticidal to antimicrobial and nematicidal activity. In this study, we evaluated the nematicidal activity of *Photorhabdus luminescens subsp*. *sonorensis*, the symbiont of an Arizona native *Heterorhabditis* species. This bacterium represents a novel source of bioactive molecules with potential applications in agriculture. Specifically, we assessed the bioactivity of metabolites produced by this bacterium two important plant-parasitic nematode species: the root- knot nematode, *Meloidogyne incognita*; and the *Poa annua* stem gall nematode, *Anguina pacificae*. Bioassays showed that *Photorhabdus l. sonorensis*' secondary metabolites have potent antagonistic activity, achieving complete mortality in *in vitro* assays. Moreover, in planta assays showed a reduction on gall formation for both plant-parasites. The composition of these extracts was analyzed by TLC, HPLC-UV, and HPLC-MS. Results of this study are herein presented and further discussed.

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Link between *Bursaphelenchus xylophilus*-associated bacteria and endophytic microbial community of *Pinus* with Pine Wilt Disease

DIOGO NEVES PROENÇA[1], PAULA V. MORAIS[1,2]

[1] *IMAR-CMA & CEMUC, University of Coimbra, Coimbra, Portugal*
[2] *Department of Life Sciences, University of Coimbra, Coimbra, Portugal*

Pine wilt disease (PWD), a major illness of several *Pinus* species, native to North-America, has spread into Asia and recently into Europe (Portugal and Spain). *Bursaphelenchus xylophilus*, the pinewood nematode (PWN), is considered the only causative agent of PWD. It has been proposed that PWD is a complex disease induced by both PWN and the bacteria it carries. This work aimed to assess the microbial community, associated with the PWN and within the host-plant, in order to elucidate the link between both community structures. Cultivation methods were used for bacteria carried by PWN from Portugal and USA. Cultivation and molecular methods (DGGE, Illumina) were used to assess the endophytic microbial community of pine trees from Portugal. Both bacterial community structures, by cultivation and molecular methods, share the class Gammaproteobacteria as the most abundant. The major bacterial species associated with the nematodes differed according to the forest area and none of the isolated bacterial species was found in all different forest areas studied. However, strains from the genus *Pseudomonas* were found associated with PWN from all countries. Moreover, bacteria associated with the PWN were able to produce siderophores, proteases and lipases and 21 of these strains showed nematicidal activity towards PWN. Bacteria belonging to the classes Gammaproteobacteria, Betaproteobacteria, Bacilli, Alphaproteobacteria and Flavobacteriia were identified associated with PWN as well as part of the endophytic microbial community of *P. pinaster*. Furthermore, by using DGGE and Illumina was possible to detected 24 additional classes comparatively to cultivation methods. In conclusion, considering the diversity of the nematode-associated bacteria and their presence in endophytic microbial community we cannot suggest an active role of the bacteria in PWD.

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Targeting the *Wolbachia* endosymbiont for human filarial nematode diseases and other insect-borne pathogens

BARTON E. SLATKO[1], ASHLEY N. LUCK[1], JEREMY M. FOSTER[1] AND MARK J. TAYLOR[2]

[1] *New England Biolabs, 240 County Road, Ipswich MA 01938*
[2] *Liverpool School of Tropical Medicine, Liverpool, L3 5QA,UK*

Most human filarial nematode parasites and arthropods are hosts for the *Wolbachia* endosymbiont. These nematodes (the causative agents of lymphatic filariasis and onchocerciasis (river blindness)) are insect vector borne. More than 150 million people in more than 80 countries are infected, and over 1 billion people are at risk. Current treatments for these diseases are effective in killing the juvenile microfilariae but have poor adult worm (macrofilaricidal) activity. As fertile adults can survive in human hosts for 10-15 years, treatments must be administered annually or semi-annually on a community-wide basis for many years to break the infection cycle. Global parasite elimination is hampered by this challenge and problems of adverse events in areas co-endemic with another filarial nematode parasite, Loa loa and by the risk of emerging drug resistance. Laboratory and human trials show that *Wolbachia* depletion by antibiotics (e.g. doxycycline) kills adult worms, blocks embryogenesis, microfilarial output and worm development and improves clinical pathology. *Wolbachia* thus represent a promising drug target for filariasis control. The mission of the worldwide Anti-*Wolbachia* consortium (A.WOL), initially funded by the Bill and Melinda Gates Foundation, is to identify new anti-*Wolbachia* drugs and regimens which are short and safe for target populations (Mass Drug Administrations) and to refine regimens of existing antibiotics suitable for a more restricted use. Using genomics and screening of compounds for anti-*Wolbachia* activity has provided several hundred potential compounds from small molecule library screening and repurposed drugs. In addition to filariasis control, *Wolbachia* may be utilized to reduce or eliminate mosquito populations due to conditional reproductive sterility (cytoplasmic incompatability), reducing insect-borne pathogen exposures not only to filarial diseases, but to other pathogens such as malaria, Dengue, Chikungunya, Yellow fever and West Nile viruses. Thus the *Wolbachia* endosymbiont, offers great potential for elimination of a wide-variety of devastating human diseases.

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The epic friendship of *Steinernema* nematodes and their bacterial symbionts

S. PATRICIA STOCK

Dept. Entomology, University of Arizona

Steinernema nematodes are able to exploit a specific niche: the nutrients available in immature stages of soil dwelling insects, through their symbiotic association with Gram-negative *Xenorhabdus* bacteria. Together nematodes and bacteria form an insecticidal complex that efficaciously kills a wide range of insect hosts, spanning across many soil-dwelling insect species. All known *Steinernema* spp. are mutualistically associated with a single species of *Xenorhabdus*. However, from the bacteria perspective a single *Xenorhabdus* species may have multiple *Steinernema* species as its hosts. A critical component of *Steinernema*'s life cycle is the transmission of the specific *Xenorhabdus* symbiont between generations through successful symbiont colonization. In this respect, a few studies suggest *Steinernema* nematodes have varying degrees of specialization for their symbionts. Moreover, it has been shown that non-cognate *Xenorhabdus* species can have adverse effects on nematode fitness. In this presentation I will summarize research conducted in my laboratory placing emphasis on the development, maintenance and evolution of the relationships between *Steinernema* nematodes and their symbiotic bacteria.

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Session Chairs: Nathalie Gontier[1], Jan Sapp[2]
Chair affiliations: [1]Universidade de Lisboa, Portugal
[2]York University, Canada

Reticulate evolution before and after the modern synthesis: historical and epistemological perspectives and wider applications beyond traditional fields

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Network-thinking: a complementary perspective to address the compelling epistemological and biological challenges raised by reticulate evolution

ERIC BAPTESTE

19 quai Saint Bernard, UPMC Bat A, UMR 7138, Paris 75005 France

Biological objects (from genes to genomes and holobionts) are composite entities, made of interacting heterogeneous parts; often brought together by reticulate processes. Describing the evolution of such complex objects, in particular the association, stabilisation, and transformation of biological elements resulting in novel higher level structures requires the developments of network-based analytical tools and of increasingly flexible representations of life's history. In order to reach this conclusion, I will introduce some conceptual challenges raised by biological data and recent discoveries from microbiology and virology, and explain how these challenges encourage to expand the framework of evolutionary analyses through the use of sequence similarity networks and bipartite graphs.

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The symbiogenic superorganism concept. An old new problem for the neo-Darwinian synthesis?

FRANCISCO CARRAPIÇO

Centro de Ecologia, Evolução e Alterações Ambientais e Centro de Filosofia das Ciências, Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, 1746-016 Lisboa, Portugal

The concept of superorganic evolution was first introduced into the scientific literature by Herbert Spencer in 1876. Although the term ‘superorganism’ was not used explicitly, this would imply a new approach to the classical concept of organism. In 1911, William Morton Wheeler, compared the ant society to an organism when observing the biology and social behavior of these insects in colonies. However, it was only in 1928 that he referred to the ant colony as a superorganism. Based on these ideas and further works, we have introduced the concept of symbiogenic superorganism applied to new entities or consortia formed by the integration of individual organisms. This concept includes four main principles: 1. it is composed of different species of organisms living together, which work towards a common goal; 2. the new entity is a polygenomic one, in which the different genomes operate together in a complementary and synergistic way for the whole; 3. the parts and units of this entity modify themselves qualitatively, compared to the same units when isolated; 4. the final outcome of the synergy is not the mere qualitative and/or quantitative sum of the units, which constitute the consortium, acquiring new collective synergies and characteristics. This reality is widespread in nature and well exemplified by *Azolla*, an aquatic pteridophyte constituted by the association of two type of prokaryote organisms (cyanobacterium and bacteria) living symbiotically inside the leaf cavity of the fern (host). All these facts challenge the traditional neo-Darwinian approach to understanding the organism concept, reinforce the principle that eukaryotes are not entities genetically unique and that the individual must be seen as a complex biological ecosystem, composed of multiple interdependent parts living symbiotically. This new perspective allows for a better understanding of the web of life in our planet and beyond.

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Modeling Symbiosis: A multiset model of multi-species evolution

LUÍS CORREIA[1], ANTÓNIO MANSO[1,2]

[1] *Laboratory of Agent Modelling (LabMAg), Department of Informatics, Faculty of Science, University of Lisbon, Lisbon, Portugal*
[2] *Tomar Polytechnic Institute (IPT), Tomar, Portugal*

This talk presents Symbiogenetic MuGA (SMuGA), an integration of symbiogenesis with the Multiset Genetic Algorithm (MuGA) to solve large binary difficult problems. The co-evolutionary model used in SMuGA evolves two species: hosts that represent a solution to the problem, and parasites that represent part-solutions. The novelty of the approach is the varying length of parasites along the evolutionary process. Additionally, it also allows multiple parasites to collaborate with a single host. To improve efficiency, we introduced proxy evaluation of parasites, which saves fitness function calls and exponentially reduces the symbiotic collaborations produced. Another novel feature consists of breaking the evolutionary cycle into two phases: a symbiotic phase and a phase of independent evolution of both hosts and parasites. SMuGA was tested in optimization of a variety of deceptive functions, with results one order of magnitude better than state of the art symbiotic algorithms. This allowed to optimize deceptive problems with large sizes, and showed a linear scaling in the number of iterations to attain the optimum in problems where some decomposition is possible. Future developments of SMuGA include the definition of new genetic operators that operate in parasites and the introduction of mechanisms to preserve genetic diversity of the parasite population. With those improvements we foresee the increased ability of SMuGA to optimize problems with very large genomes and the application of the model to other types of functions.

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Intracellular endosymbiont selection contributes to *Drosophila* adaptation to viral infection

VITOR G. FARIA[1], ÉLIO SUCENA[2]

[1] *Instituto Gulbenkian de Ciência, Apartado 14, 2780-901 Oeiras, Portugal*
[2] *Universidade de Lisboa, Faculdade de Ciências, Departamento de Biologia Animal, Edifício C2, Campo Grande, 1749-016 Lisboa, Portugal*

Bacteria of the genus *Wolbachia* are intracellular symbionts of many animal species. In *Drosophila*, *Wolbachia* has been shown to confer protection to RNA virus infection in a strain-dependent manner. Through experimental evolution, we have selected an outbred *Wolbachia*-infected population of *D. melanogaster* for increased resistance to DCV, a natural viral pathogen. Whole-genome sequencing of this population, upon 20 generations of selection, revealed that a *Wolbachia* sub-strain was fixed. Moreover, we show that challenged inter-population hybrids carrying either *Wolbachia* variant differ in their fitness, confirming the adaptive value to the host of selected endosymbiont. Finally, we re-assess host genome evolution upon *Wolbachia* clearance and DCV infection over 20 more generations. These findings demonstrate that the presence of protective endosymbiont plays a role in shaping the host genome and its own evolution may have a profound effect on host adaptation.

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Book launch: reticulate evolution: Symbiogenesis, lateral gene transfer, hybridization, and infectious heredity. Springer, Dordrecht (2015)

NATHALIE GONTIER

Applied Evolutionary Epistemology Lab, Centre for Philosophy of Science, Universidade de Lisboa, Campo Grande, C4 3.12, Lisbon, PT

Written for non-experts, this volume introduces the mechanisms that underlie reticulate evolution. Chapters are either accompanied with glossaries that explain new terminology or timelines that position pioneering scholars and their major discoveries in their historical contexts. The contributing authors outline the history and original context of discovery of symbiosis, symbiogenesis, lateral gene transfer, hybridization or divergence with gene flow, and infectious heredity. By applying key insights from the areas of molecular (phylo)genetics, microbiology, virology, ecology, systematics, immunology, epidemiology and computational science, they demonstrate how reticulate evolution impacts successful survival, fitness and speciation. Reticulate evolution brings forth a challenge to the standard Neo-Darwinian framework, which defines life as the outcome of bifurcation and ramification patterns brought forth by the vertical mechanism of natural selection. Reticulate evolution puts forward a pattern in the tree of life that is characterized by horizontal mergings and lineage crossings, making the “tree of life” look more like a “web of life.” On an epistemological level, the various means by which hereditary material can be transferred horizontally challenges our classic notions of units and levels of evolution, fitness, modes of transmission, linearity, communities, and biological individuality.

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Session: Reticulate evolution before and after the modern synthesis
Presentation type: Keynote
Name, Contact: Guerrero Ricardo, rguerrero@iec.cat

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From the cell to the ecosystem: The physiological evolution of symbiosis

RICARDO GUERRERO[1,2], MERCEDES BERLANGA[2]

[1] *Academia Europaea*
[2] *University of Barcelona*

The small size, ubiquity, metabolic versatility and genetic plasticity of microorganisms allow them to quickly adapt to unfavorable and/or changing environmental conditions. Bacteria and archaea live in complex multispecies communities. Living organisms constantly interact with their habitats, selectively taking up compounds from their surroundings and excreting metabolic products and thus modifying their environment. The consumption of resources and the formation of metabolic products by spatially separated microbial populations constitute the driving force that leads to gradient formation. By the cell theory, the cellular membrane is the first and significant gradient, that that differentiates the "I" from the "Other". It has been suggested that communication and cooperation, both within and among bacterial species, have produced emergent properties that give a selective advantage. Bacteria do not function as individuals; rather, the vast majority of bacteria in natural and pathogenic ecosystems live in aggregates referred to as biofilms. These bacterial surface-associated communities are attached to solid substrata. Such metabolically integrated consortia consisting of multiple species can adopt specific spatial configurations. Indeed, the bacterial consortia of biofilms reach levels of complexity nearing those of multicellular eukaryotes. Microbial consortia functions were maintained throughout the evolution, from the first ecosystems, such as microbial mats, to the recently (geologically speaking) intestinal tract of animals, such as the xylophagous insects. The autopoietic unit, whether a cellular biont (minimal autopoietic unit) or a holobiont (integrated biont organisms, i.e., animals or plants, with all of their associated microbiota), is capable of self-maintenance by sensing the environment and is able to adapt and evolve. Complex autopoietic units acquire novel properties when the assembly of their components results in higher functional-structural complexity. However, autopoiesis alone, is a necessary but not a sufficient condition for the evolution of life.

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A symbiotic view of biological individuality

VECCHI DAVIDE

Centre for Philosophy of Sciences of the Faculty of Sciences University of Lisbon, Campo Grande, Edifício C4, Lisbon, 1749-016, Portugal

Matter is organised hierarchically, with components creating higher level entities in a recursive way. Herbert Spencer proposed that evolution can be characterized by two principles: continuous multiplication of parts (i.e., instability of the homogeneous) and progressive integration (i.e., stability of the heterogeneous). Living matter is no exception. Teilhard de Chardin considered "conjugation" (i.e., the merging of lineages) and association (i.e., symbiosis) as "elemental movements of life". Whether accidental or not, many evolutionary transitions were the result of processes of integration, most assuredly eukaryogenesis, but possibly also the emergence of cells. So, assuming that symbiosis is an evolutionarily significant process, what does the fact of symbiosis imply for the notion of biological individual? Symbiosis can be characterised as an aggregative force whereby physiologically and reproductively autonomous biological individuals progressively associate by sharing, for example, a metabolic fate. The strength of the association varies from a partial, reversible and transitory association to an obligate, irreversible and permanent one. The strength of the association is inversely proportional to the degree of autonomy of the biological individuals involved. Symbiosis therefore poses a potential problem for those accounts of biological individuality that are based on a static characterisation of autonomy. In fact, the existence of various degrees of physiological and reproductive integration implies the relinquishment of biological autonomy on the part of the host and symbiont. In this talk I will show in what sense the connected concepts of autonomy, autopoiesis and organisational closure are challenged by evidence for symbiosis. The fact of symbiosis demonstrates the interpenetration between living system and environment as well as the ontogenetic and phylogenetic negotiability of their relationship. The fact of symbiosis implies that transitional individuals, mutualistic symbioses and the creation of holobionts are common and evolutionarily significant biological phenomena. But are they?

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Untangling the evolutionary history of the human microbiome using Neanderthal dental calculus: Cultural and environmental impacts on human health, disease, and evolution

LAURA S WEYRICH[1], KEITH DOBNEY[2], AND ALAN COOPER[1]

[1] *University of Adelaide, Australia*
[2] *University of Aberdeen, UK*

Interpreting the evolutionary history of bacterial communities within the human body (microbiome) is key to understanding multiple aspects of health and disease, and elucidating mechanisms that underlie bacterial and human co-evolution. Although research once suggested that the human microbiome evolved in accordance with the hominid evolutionary tree, recent evidence has indicated that the human microbiome underwent significant changes after the split between human and chimpanzee lineages. To examine this theory in greater detail, we recovered ancient bacterial DNA within dental calculus (calcified dental plaque) from Neandertals, ancient and present-day humans, and greater apes to determine the evolutionary history of the hominid microbiome. Similar bacterial community structures were detected in all non-agriculturalist specimens (Neandertals, chimpanzees, African and European hunter-gatherers), revealing the existence of a once shared hominid microbiome. In contract, a marked change was observed in the oral microbiome when humans adopted agriculture in both Europe and Africa; individuals from both continents exhibited similar core and highly abundant bacterial species, despite the vastly different cultivars and timing. Further alterations to the human oral microbiome were observed after the Industrial Revolution, and again during the coming of the modern age, revealing additional cultural and environmental factors that can significantly impact the human microbiome, and alter the evolutionary signal associated between bacteria and humans. Consequently, essentially all present-day humans possess an evolutionarily recent oral microbiome that was introduced after the introduction of farming Europe, nearly 7,500 years ago. These ancient and historical samples providing timing and reveal cultural and environmental factors that altered the relationship between humans and their microorganisms, which may have significant health consequences in the modern world.

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Session Chairs: Manuela Giovannetti
Chair affiliations: Università di Pisa, Italy

Symbiont-based improvement of plant nutraceutical value

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Arbuscular mycorrhizal fungi and rhizobacteria for the improvement of the nutraceutical values of crops

GRAZIELLA BERTA, ELISA GAMALERO, GUIDO LINGUA

Università del Piemonte Orientale

Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting bacteria (PGPB) can increase the growth and yield of major crops, and improve the quality of flower parts, fruits, seeds and leaves. Results on the effects of inoculation with AMF and/or PGPB, on the quality and yield of a number of crops (tomato, strawberry, mais, saffron) will be presented. The experiments were performed in controlled conditions and/or in the field, in conditions or not of reduced chemical inputs.We showed that the microorganisms used enhanced flower and fruit production of tomato and strawberry, also affecting their quality, considering for this purpose concentration of sugars, organic acids and vitamins (ascorbic and folic acid),higher in AM and PGPB inoculated plants. In tomato, none of the treatments affected fruit lycopene content, whilst in strawberry attention was paid to fruit anthocyanin content that resulted increased. Anthocyanins, phenolic compounds that are products of the secondary metabolism in plants, are important for both the food industry and human nutrition. The grain composition of maize plants inoculated with AMF and PGPB was assessed. The bacterial inoculum increased grain starch content, especially the digestible components, whereas AMF enhanced protein, especially zein, content.For saffron, experiments in the field with AMF and PGPB showed a significant increase of the bulb diameter, to which corresponded a higher flower production. Saffron samples (stigmas) from the different treatments were analysed by HPLC-DAD-MS technique. Typical components of saffron, among which the crocine congeners, were detected. The relative intensities of the chromatographic signals were shown to depend on the beneficial soil bacteria and AMF. Our results show that the inoculation of crops with selected PGPB and AMF used as biofertilizers can improve their yield and quality also allowing, at least for strawberry and tomato, a decrease of chemical inputs.

Impact of mycorrhiza on health- and flavour-related food quality in tomato and moringa

MARCO COSME

Freie Universität Berlin, Dahlem Centre of Plant Sciences, Functional Biodiversity, Berlin, Germany and Leibniz-Institute of Vegetable and Ornamental Crops, Großbeeren, Germany

Arbuscular mycorrhizal fungi (AMF) enhance the mineral nutrition, growth, and resistance of crops, but their effect on the edible portions of plants is less understood. Several studies were carried out to investigate the impact of AMF on health- and flavour-related traits in edible tissues of the horticultural crops tomato and moringa. In tomato, AMF increased the nutrient quality of fruits for most analyzed nutrients. The tomato antioxidant capacity and the amounts of carotenoids and of several flavour compounds were significantly higher in AMF-inoculated plants compared with non-AMF controls. Although some genes encoding putative allergens were up-regulated in tomato fruits of AMF-inoculated plants, skin reactivity tests with a group of tomato-allergic subjects showed no differences. In edible leaves of moringa, AMF-inoculated plants harboured lower amounts of carotenoids, but the levels of glucosinolates were enhanced. Although it is premature to declare that these changes have manifested impact on the nutrition and health of consumers, our results suggest that AMF could be a suitable tool for improving food quality in horticultural crops.

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Apocarotenoids from arbuscular mycorrhizal roots - persistent mysteries

THOMAS FESTER

Helmholtz-Centre, Germany, Permoserstraße 15 Leipzig, 04318, Germany

Metabolite profiling analyses demonstrate that plants are reacting to external factors with complex shifts in the steady state levels of large numbers of metabolites. Such shifts are particularly pronounced in the case of abiotic stress with clear shifts even in field studies. Colonization of plant roots by arbuscular mycorrhizal fungi, in contrast, results in relatively weak shifts in metabolite levels, with the notable exception of the accumulation of carotenoid degradation products. Such compounds are not detected in colonized roots of all plant species. In some cases, however, they are dominant constituents of the colonized root's spectrum of secondary metabolites, with a particular predominance in monocotyledonous plants. Regardless of the occurence of such compounds, the activation of carotenoid biosynthesis in the roots of mycorrhizal plants seems to be a universal phenomenon in all plant species examined. Details of the accumulation (kinetics and location) of apocarotenoids together with data from plants with reduced activity of enzymes involved in this accumulation seem to support some connection to the degradation of fungal structures, although precise mechanisms for such a connection remain elusive. Conclusion and significance: A common feature of the arbuscular mycorrhizal interaction (i.e., occuring in all plant species examined) resulting in massive metabolic shifts at least in some cases and connected to conspicuous cytological changes is still poorly understood.

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Health-promoting phytochemicals in artichoke and tomato as affected by beneficial mycorrhizal symbionts

CRISTIANA SBRANA[1], LUCIANO AVIO[1], MANUELA GIOVANNETTI[2]

[1] *CNR, Institute of Agricultural Biology and Biotechnology, Italy*
[2] *University of Pisa, Dept Agricultre, Food and Environment, Italy*

The production of plant food rich in health-promoting compounds is affected by crop genotype, agronomic management and soil quality, including the occurrence of beneficial plant symbionts, such as arbuscular mycorrhizal fungi (AMF), which promote plant stress tolerance, mineral nutrition, activity of antioxidant enzymes and secondary metabolites content. Globe artichoke flower heads, traditional components of the mediterranean diet, are one of the richest dietary sources of bioactive polyphenols, in particular hydroxycinnamates (mono- and di-caffeoylquinic acids) and flavonoids (apigenin and luteolin derivatives), high-quality inulin, fibres and minerals. Moreover, artichoke leaf extracts show hypocholesterolhemic and choleretic properties. In our work, globe artichoke plants, nursery-inoculated with the AMF species *Rhizophagus intraradices* and *Funneliformis mosseae*, showed larger flower heads phenolic content and antioxidant activity, both before transplant and after two years' growth in the field, compared with control plants. Clinical evidences suggest that tomato fruits are able to reduce risks of certain types of cancers and cardiovascular diseases, due to their richness in antioxidant metabolites. Tomato plants nursery-inoculated with the AMF species *Rhizophagus intraradices* showed higher mineral content and enhanced levels of lycopene in fruits, compared with controls. Since some polyphenols may display estrogenic/antiestrogenic or genotoxic activity, tomato fruit safety was assessed by testing their *in vitro* genotoxic and estrogenic/antiestrogenic activities. Results showed that symbiotic-related modifications of plant secondary metabolism did not involve the production of unsafe compounds, since no *in vitro* genotoxic effects were detected. Interestingly, lipophilic tomato extracts strongly inhibited the E2 - human erα binding, showing significantly higher antiestrogenic power, compared with control extracts. AMF inoculation induced higher nutritional and nutraceutical value in globe artichoke and tomato without the production of unsafe compounds, and may represent a sustainable agriculture method to improve plant nutrient availability, reducing the need of chemical fertilisers and pesticides, and produce safer and higher quality food crops.

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Session: Symbiont-based improvement of plant nutraceutical value
Presentation type: Keynote
Name, Contact: Zubek Szymon, szymon.zubek@uj.edu.pl

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Interactions of medicinal plants with arbuscular mycorrhizal fungi

SZYMON ZUBEK

Institute of Botany, Jagiellonian University, Kopernika 27, 31-501 Kraków

The aims of the presented studies were to investigate both the impact of AMF on selected species of medicinal plants and the influence of medicinal plants under cultivation on these microorganisms.The laboratory experiments showed that AMF influence the concentrations of thymol and anthraquinone derivatives in *Inula ensifolia* and *Hypericum perforatum*, respectively, as well as phenolic acids and flavonoids in *Viola tricolor*. The diverse effects of different AMF on the concentrations of these chemical compounds were observed. Some of the AMF species applied increased the concentration of these metabolites in plants. The enhanced production of secondary metabolites in the plants might be due to improved mineral nutrition by AMF and/or a result of general plant defense reaction to fungal colonization. Regardless of the mechanisms, these AMF species may be used to improve the quality of herbal materials. The field experiments revealed that the long-term monocultures of mycorrhizal species (*H. perforatum, Levisticum officinale, Mentha ×citrata subsp. citrata, Thymus vulgaris*) had no effect on the level of AMF propagules, however, had an impact on AMF species composition. The cultivation of nonmycorrhizal species, *Chelidonium majus*, reduced the number of AMF propagules in soils, which can be detrimental to medicinal plants that are dependent on mycorrhizal symbiosis and would be cultivated in the future in this area. The aim of the second stage of field experiments was to investigate the combined effect of mineral and organic fertilization and three mycorrhizal plant species under cultivation (*Lavandula angustifolia, Melissa officinalis, Salvia officinalis*) on the AMF diversity and abundance of their propagules. It was found that the effect of the type of fertilization on these microorganisms depends on the plant species. Furthermore, mineral fertilizers used in moderate amounts did not have a negative impact on AMF, while having a positive effect on species diversity.

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Session Chairs: Flore Zélé[1], Sara Magalhães[2], Maria J.Pozo[3]
Chair affiliations: [1]Universidade de Lisboa, Portugal; [2]Universidade de Lisboa, Portugal
[3]Consejo Superior de Investigaciones Científicas, Spain

Symbionts in multipartite interactions: Ecology, evolution and regulatory mechanisms

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Session: Symbionts in multipartite interactions
Presentation type: Poster
Name, Contact: Baumgarten Sebastian, sebastian.baumgarten@kaust.edu.sa

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The genome of *Aiptasia*, a sea anemone model for coral biology

SEBASTIAN BAUMGARTEN[1], OLEG SIMAKOV[2]+, LISL Y. ESHERICK[3], YI JIN LIEW[1], ERIK M. LEHNERT[3], CRAIG T. MICHELL[1], YONG LI[1], ELIZABETH A. HAMBLETON[2], ANNIKA GUSE[2], MATT E. OATES[4], JULIAN GOUGH[4], VIRGINIA M. WEIS[5], MANUEL ARANDA[1], JOHN R. PRINGLE[3], CHRISTIAN R. VOOLSTRA[1]

[1] *Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal 23955, Saudi Arabia*
[2] *Centre for Organismal Studies, Heidelberg University, 69120 Heidelberg, Germany*
[3] *Department of Genetics, Stanford University School of Medicine, Stanford, California 94305, USA*
[4] *Department of Computer Science, University of Bristol, Bristol BS8 1UB, United Kingdom*
[5] *Department of Integrative Biology, Oregon State University, Corvallis, Oregon 97331, USA*
+ Current address: Okinawa Institute of Science and Technology, Okinawa 904-0495

The most diverse marine ecosystems, coral reefs, depend upon a functional symbiosis between a cnidarian animal host (the coral) and intracellular photosynthetic dinoflagellate algae. The molecular and cellular mechanisms underlying this endosymbiosis are not well understood, in part because of the difficulties of experimental work with corals. The small sea anemone *Aiptasia* provides a tractable laboratory model for investigating these mechanisms. Here we report on the assembly and analysis of the *Aiptasia* genome, which will provide a foundation for future studies and has revealed several features that may be key to understanding the evolution and function of the endosymbiosis. These features include genomic rearrangements and taxonomically restricted genes that may be associated with the adoption of a symbiotic lifestyle, aspects of host dependency on alga-derived nutrients, a novel and expanded cnidarian-specific family of putative pattern-recognition receptors that might be involved in the animal-algal interactions, and extensive lineage-specific horizontal gene transfer into both the alga and the host. Extensive integration of genes of prokaryotic origin, including genes for antimicrobial peptides, presumably reflects an intimate association of the animal-algal pair also with its prokaryotic microbiome.

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Genome-wide survey of polyketide synthases in the symbiotic dinoflagellate *Symbiodinium minutum*

GIRISH BEEDESSEE, KANAKO HISATA, SUTADA MUNGPAKDEE, NORI SATOH, EIICHI SHOGUCHI

Marine Genomics Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan

Dinoflagellates are important unicellular eukaryotes found in both marine and freshwater environments and are rich sources of unique bioactive secondary metabolites of polyketide origin. Much is known regarding the biosynthesis of polyketides from terrestrial or freshwater organisms and it is only in the recent years that polyketides of dinoflagellates are being investigated. The symbiotic marine dinoflagellate *Symbiodinium* sp. is a dominant symbiont in corals and despite the availability of the 616 Mbp draft genome of *Symbiodinium minutum* Clade B1, no gene cluster for polyketide synthases (PKS) has been reported. To find enzymes involved in polyketide synthesis namely ketosynthase (KS), acyl transferase (AT) and acyl carrier protein (ACP), we surveyed ~42,000 protein-coding genes of *S. minutum* genome and identified 31 KS genes to be expressed as several functional domains within a single protein. Additionally, a maximum likelihood phylogenetic analysis of KS domain formed a clade placing well within the protist Type I PKS when compared with other dinoflagellates and eukaryotes confirming their Type I PKS nature. The predicted proteins had functionally important amino acid residues required for catalysis (cysteine, histidine and lysine) within the DTACSS-motif of KS domain in 28 out of 31 sequences. Interestingly, the largest enzyme is likely to be expressed as a hybrid NRPS/PKS assembly of 10,601 amino acids containing 3 NRPS modules divided by 2 PKS modules and ending with a thioesterase (TE) domain. Our genomic survey demonstrate that the minimal set of catalytic domains needed to produce polyketides namely KS, AT and ACP along with other optional domains are present in *S. minutum*. Metabolite profiling using NanoLC-MS revealed the presence of the known zooxanthellamide D, a polyhydroxy amide polyketide of 1050.5657 [M+H]+. Our findings support the modular nature of Type I PKS in *Symbiodinium* in contrast to previous reports of monofunctional Type I PKS among other dinoflagellates.

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Can bacterial symbionts push galling aphid over the speciation cliff?

LIHI AMIT, RACHEL BEN-SHLOMO, ELAD CHIEL

Department of Biology & Environment, University of Haifa-Oranim, Israel

The gall-forming aphids *Slavum wertheimae* and *Forda riccobonii* (Hemiptera: Pemphigidae) are both specific to their host, *Pistacia atlantica* (Sapindales: Anacardiaceae). The distribution of *P. atlantica* in Israel is mosaic, resulting in patches of populations from the north to the south, which leads to fragmented populations of the gall-forming aphids as well. Former studies revealed genetic differentiation between the populations of the gall-forming aphids, suggesting cryptic allopatric speciation, while *P. atlantica* show no such differences. Is geographic distance the only variable in this equation?In this study we tested the hypothesis that bacterial symbionts, such as *Wolbachia*, that are known as insects' reproductive manipulators play a role in the speciation of the galling aphids. By means of diagnostic PCR we screened for the presence of 9 known aphids symbionts: *Wolbachia*, *Rickettsia*, *Cardinium, Spiroplasma, Serratia, Regiella, Hamiltonella, Rickettsiella* and X-type in several populations of *S. wertheimae* and *F. riccobonii* along Israel. Almost all individuals from all the populations tested of *S. wertheimae* harbored *Rickettsia*, *Cardinium* and *Spiroplasma*. In contrast, *Wolbachia* was found only in the northern populations, correlating with the galling aphid's genetic differences. In *F. riccobonii* we found high prevalence of *Cardinium* and *Rickettsia*, intermediate prevalence of *Spiroplasma* and no *Wolbachia*, in all the populations. The rest of the symbionts were not detected in any of the populations. Next, we sequenced-typed the *Wolbachia* in the northern populations of *S. wertheimae* according to the MLST protocol. We found two closely related, sequence types, both new to the database, which may indicate an ongoing speciation process of *Wolbachia*.Future research will help to clarify if the genetic differences between the populations of *S. wertheimae* are driven by *Wolbachia*, or vice versa – if the differential infection patterns are a result of geographic isolation.

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Endosymbionts mediate host-parasitoid coevolution and alter parasitoid community composition

CHRISTOPH VORBURGER

Institute of Integrative Biology, ETH Zurich, 8092 Zürich, Switzerland & Eawag, Swiss Federal Institute of Aquatic Science & Technology, 8600 Dübendorf, Switzerland

Many symbionts are passed on from parent to offspring. Vertical transmission couples host and symbiont fitness and has thus facilitated the evolution of defence mechanisms by which symbionts protect their hosts against parasites. This challenges existing theory on host-parasite interactions. Using models that incorporate effects of symbionts, we identify several factors that are important in determining the dynamics of host-parasitoid coevolution when mediated by symbionts, such as the specificity of symbiont-conferred protection, the costs associated with harbouring symbionts, or their rate of horizontal transmission. We are in the process of studying these factors empirically in a model system comprising the black bean aphid, *Aphis fabae*, its defensive symbiont *Hamiltonella defensa*, and the parasitoid *Lysiphlebus fabarum*. The results so far indicate that symbionts behave such that they do indeed play a crucial role by altering the reciprocal selection between hosts and parasitoids, leading to coevolutionary dynamics that are not observed in their absence. I also show that the strong specificity of symbiont-conferred resistance leads to marked differences in the composition of parasitoid communities supported by protected and unprotected aphids in the field.

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Host preference of symbiotic gastropods (Eulimidae): experimental data

YURI KANTOR

A.N. Severtson Institute of Ecology and Evolution

The most complex types of cohabitation occur on coral reefs: in such ecosystems symbiotic species form a variety of interactions based on trophic and topical relations. Associations between echinoderms and symbiotic organisms are convenient models for studying intra- and interspecific relationships, as well as various aspects of the biology of the symbionts. The family Eulimidae is a diverse group of highly specialized marine gastropods which exclusively inhabit echinoderms. Unfortunately many eulimids species were described without their hosts and host specificity as well as preferences remain unknown. For several years we are studying eulimids in Central Vietnam, especially in the Nha Trang Bay, with special emphasis on symbiont-host interactions. The aim of this study was to test in experiments whether the snails are able to detect their host using chemoreception.Laboratory experiments were conducted for identification of host preference in *Annulobalcis albus, Annulobalcis vinarius* and *Annulobalcis maculatus* from different species of crinoids. We used Y-tube of plexiglas that allows to investigate the effect of chemical factors and to exclude the others. Water from different sources (from aquarium, containing crinoids, or similar sea-water filled aquarium without crinoids) was supplied to distal parts of Y-tube. Mollusc was placed to the middle of the common tube part.As results we found that almost all tested eulimids species can recognize their hosts. *Annulobalcis albus* and *Annulobalcis maculatus* more often selected the water from the aquaria with their hosts and weakly responded to water from the aquaria without any crinoids. *Annulobalcis vinarius*, which is lower host specific symbiont, preferred crinoid *Himerometra robustipinna* (main host) more frequently than other (alternative host) crinoid hosts species – *Comatella nigra* and *Stephanometra tenuipinna*, on which it was recorded less often in nature. Probably, molluscs use *Comatella nigra* and *Stephanometra tenuipinna* as a reserve hosts and colonize them in rare cases.

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Effects of starvation in *Reticulitermes grassei* symbiotic flagellate protists community

SÓNIA DUARTE[1,2], TÂNIA NOBRE[3], PAULO A.V. BORGES[2], LINA NUNES[1,2]

[1] *LNEC (Structures Department, Lisbon)*
[2] *cE3c (Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group, Azores)*
[3] *ICAAM (Évora University)*

Subterranean termites' ability to digest lignocellulose relies not only on their digestive tract physiology, but also on the symbiotic relationships established with flagellate protists and bacteria. This partnership has been very successful due to their co-adaptation ability and efficacy in solving the needs of involved organisms: this tripartite symbiosis may have reached a stable, though dynamic, equilibrium. The flagellate protist community living inside termites is rather diverse, and a strong division of labour among them is likely to exist to accomplish the intricate process of lignocellulose digestion.The objectives of this work were to: 1) investigate the response of the flagellate protists community to starvation; and 2) evaluate the recovery capacity of the flagellate protists community after a starvation process. Termites from the same colony were submitted to different starvation levels. A positive control (no starvation) and a negative control (starvation until death) were also set.It was possible to identify a more susceptible group of flagellate protists, which were only present in the positive control and termites submitted to less than 5 days of starvation: *Trichonympha agilis, Dinenympha fimbriata* and *Pseudotrichonympha* spp.The most resilient taxa were *Microjoenia* spp., *Dinenympha gracilis* and *Pyrsonympha* spp. These protists were present in negative controls all through the starvation period. The average abundance of the first two protists referred decreased in the termites submitted to more than 5 to 7 days of starvation. However, for *Pyrsonympha* spp. it was observed an increase or constancy of the average abundance, despite the starvation time.In order to maintain the holobiont alive, the most resilient groups of protists should be able to perform the bulk of lignocellulolytic activities. The results, although preliminary, may be valuable either for innovative termite control strategies targeting the flagellate protists as for other research areas with potential spin-offs of industrial use.

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Insect symbionts mediate indirect interactions in aphid communities

ENRIC FRAGO[1], DIRK SANDERS[2], RACHEL KEHOE[2] , FRANK VAN VEEN[2] , AILSA MC LEAN[3] , CHARLES GODFRAY[3] , MARCEL DICKE[1] , RIETA GOLS[1]

[1] *Wageningen University*
[2] *Exeter University*
[3] *Oxford University*

It has long been known that insect mutualistic symbioses play an important role in insect-plant interactions. In the last decade, it has become apparent that facultative symbionts (i.e. not required for host survival) can be important mediators of interactions between insects, their host plants and their natural enemies. Little is known, however, about the role of symbionts in mediating indirect (i.e. separated by more than one trophic link) species interactions. In microcosm laboratory experiments involving three aphid and three parasitoid species, we demonstrated that the symbiont Hamiltonella defensa can have important implications for the long term dynamics and the diversity of this aphid community. In the filed, however, the presence of the symbiont in a similar community didn't affect its dynamics, but the density of the different species, provably due to changes in aphid abundance and plant nutritional quality. Our study shows for the first time that symbionts can be important players that affect plant and parasitoid-mediated indirect interactions in insect communities.

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Green sea slugs: plastid symbiosis in animal cells

JAN DE VRIES, CESSA RAUCH, GREGOR CHRISTA, SVEN B GOULD

Universitätsstr. 1, Düsseldorf, NRW 40225, Germany

Elysia viridis (Gastropoda, Sacoglossa) is one of the few known sea slug species that can survive starvation long-term in the presence of stolen plastids (kleptoplasts). Recently, a detailed study on two related sister taxa, *Elysia timida* and *Elysia cornigera*, demonstrated that these two responded very differently to starvation although both fed on the same food alga, *Acetabularia acetabulum*. In this respect *E. viridis* is special, because it can feed on several different algal species and its starvation period might depend on the food source. This is a unique system within the Sacoglossa and allows analyzing the effect of the food source on the response to starvation. We collected *E. viridis* from the costal shores of Zeeland, Netherlands and from Praia de Barra, Portugal and fed them in the laboratory solely on *Codium* spec. or on *Bryopsis* spec. Slugs were then separated into different groups (dark, light, chemically blocked photosynthesis and a control starvation group) for analysis during starvation. For specimens fed on either of the two food sources the PSII quantum yield was measured. In general, specimens fed on *Bryopsis* spec. lost their functional plastids quicker and die earlier, but the importance of photosynthesis remains questionable. Furthermore, specimens fed on *Codium* spec. showed lower levels of ROS evolution, which is reminiscent of what was observed for another long-term retention species. It suggests that the algal source from which plastids are sequestered has a direct impact on the survival rate of *Elysia viridis*. Our results provide evidence that the right plastid source is as essential as the adaption of the slug biology to endure starvation.

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High endogenous levels of cytokinins differently affect the entry of beneficial root symbionts in the pea nodulation mutant E151

FREDERIQUE C. GUINEL[1], RJ NEIL EMERY[2]

[1] *Wilfrid Laurier University*
[2] *Trent University*

E151 (Pssym15), a mutant of pea (*Pisum sativum* L), exhibits unusual root and symbiotic traits. It has only a few multi-lobed nodules when in contact with nitrogen-fixing rhizobia but is highly colonized by mycorrhizal fungi. Furthermore, whereas young E151 seedlings possess few emerged lateral roots (LR) but many LR primordia, older E151 plants have a similar LR number to that of the wild-type (WT). The symbiotic traits of E151 are shared with WT pea treated with exogenous cytokinin (CK), with mutants of Lotus japonicus and *Medicago truncatula* affected in CK perception, and with tomato (*Lycopersicon esculentum*) plants treated with abscisic acid (ABA). We therefore hypothesized that E151 accumulates CK and ABA. To test this hypothesis, we extracted the hormones from roots grown for 6 days in contact with either type of micro-symbionts. Control plants were grown without any rhizobia or fungi. Purified CK analytes were quantified by HPLC-ESI MS/MS spectrometry. We found that E151 roots grown without symbionts had significantly higher CK than WT roots. Although the levels of CK nucleotides, CK ribosides, and total CK were increased, those of the CK nucleotides were predominant. Surprisingly, upon rhizobial or fungal inoculation, the CK levels decreased in E151 whereas they remained constant in WT. The decrease was significant in the presence of the fungi but not in that of rhizobia. ABA levels did not differ between the two pea lines' roots, whether they were inoculated or not. Our results suggest that the rhizobial and fungal symbionts respond differently to high endogenous CK levels. While the entry of the former into the root cortex is negatively affected, that of the fungus is promoted. This is the first evidence of a link between CK and mycorrhizal development, emphasizing the likely role of this hormone in the regulation of the agriculturally-important mycorrhizal symbiosis.

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Horizontal transfer leads to competition and loss in an unstable pea aphid multiple endosymbiont infection

ELEANOR HEYWORTH, JULIA FERRARI

University of York

Bacterial endosymbionts of insects are common, widespread and can significantly impact their host's fitness. Although many symbioses are now known to involve multiple partners, the resulting complex dynamics are still relatively unexplored. We use pea aphids (*Acyrthosiphon pisum*) to study multi-species symbiont infections. Pea aphids are infected with the obligate nutritional symbiont *Buchnera aphidicola* and up to eight vertically-transmitted facultative symbionts, making them an ideal system for exploring these interactions. We created double infections of two facultative symbionts, *Regiella insecticola* and X-type and followed their dynamics over multiple generations using qPCR to measure symbiont titre and loss. Despite multiple infections of non-obligate symbionts being common in aphid populations, the species we used showed unexpected instability when introduced. We explored the dynamics of both symbionts before and after artificial infection and discovered compelling evidence for competition between the bacterial species. This work demonstrates limitations to the spread of beneficial infections in the field, provides a potential mechanism for symbiont loss and replacement and shows the ability of endosymbiotic bacteria to react to potential competitors.

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Differential responses of the whitefly *Bemisia tabaci* symbionts to unfavorable low and high temperatures

HONG-WEI SHAN, YU-HENG LU, XIAO-LI BING, SHU-SHENG LIU, YIN-QUAN LIU

Ministry of Agriculture Key Laboratory of Agricultural Entomology, Institute of Insect Sciences, Zhejiang University

The whitefly *Bemisia tabaci* complex contains many cryptic species, of which the Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) are notorious invasive pests. In China, MEAM1 harbors an obligate primary symbiont “Candidatus *Portiera aleyrodidarum*” and two secondary symbionts, “Candidatus *Hamiltonella defensa*” and *Rickettsia* sp., whereas MED has only “Ca. *Portiera aleyrodidarum*” and “Ca. *Hamiltonella defensa*”. Both “Ca. *Portiera aleyrodidarum*” and “Ca. *Hamiltonella defensa*” are intracellular endosymbionts residing in the bacteriomes, whereas *Rickettsia* sp. has a scattered distribution throughout the host body cavity. We examined responses of these symbionts to adverse temperatures as well as survival of the host insects. After cold treatment at 5°C or 10°C or heat treatment at 35°C or 40°C for 24 hours respectively, the infection rates of all symbionts were not significantly decreased based on diagnosis PCR. However, quantitative PCR assays indicated significant reduction of “Ca. *Hamiltonella defensa*” at 40°C, and the reduction became greater as the duration increased. Compared with “Ca. *Hamiltonella defensa*”, “Ca. *Portiera aleyrodidarum*” was initially less affected in the first day but then showed more rapid reduction at days 3-5. The density of *Rickettsia* sp. was not reduced significantly at 40°C. Fluorescences *in situ* hybridization analysis verified the change of symbionts. The bacteriomes and bacteriocytes of whitefly were suffered negative effects during high temperature stress. Meanwhile, the mortality rates of the host whiteflies elevated rapidly as the duration of exposure to heat treatment increased. The differential responses of various symbionts to adverse temperatures imply complex interactions among the symbionts inside the same host insect and highlight the importance of taking the whole bacterial community into account in studies of symbioses.

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Presentation type: Oral
Name, Contact: Manzano-Marín Alejandro, alejandro.manzano@uv.es

No subfamily is perfect…: Secondary symbiont settlement, replacement and internalization in the subfamily Lachninae of aphids

ALEJANDRO MANZANO-MARÍN[1], GITTA SZABO[2], JEAN-CHRISTOPHE SIMON[3], MATTHIAS HORN[2], AMPARO LATORRE[1]

[1] *University of Valencia, Institut Cavanilles de Biodiversitat i Biologia Evolutiva. Valencia, Spain*
[2] *University of Vienna, Department of Microbiology and Ecosystem Science. Vienna, Austria*
[3] *Agrocampus Ouest, UMR 1349 IGEPP. Rennes, France*

Most aphids harbour the obligate endosymbiotic bacteria *Buchnera aphidicola*. These, endow the aphids with an expanded set of metabolic capabilities such as biosynthesis of vitamins and essential amino acids which are required for their survival. In the Lachninae subfamily of aphids, It was found that many members seemed to be always associated to a *Serratia symbiotica* secondary endosymbiont. Through whole genome sequencing, we have determined that *Cinara tujafilina* (Eulachnini tribe), *Cinara cedri* (Eulachnini tribe) and *Tuberrolachnus salignus* (Lachnini tribe) have indeed established co-obligate associations with the typical *B. aphidicola* plus *S. symbiotica*. Nevertheless, while in *C. tujafilina S. symbiotica* is extracellular, rod shape and possesses a big genome, in *C. cedri* and *T. salignus* they are intracellular, pleomorphic and have very small genomes that have apparently arose independently. We have previously proposed that a common ancient loss of the riboflavin biosynthesis pathway in the *Buchnera* from the Lachninae common ancestor, caused *S. symbiotica* to fix as an obligate endosymbiont. However, some aphids such as *Maculolachnus submacula* and *Eulachnus rileyi* have been found to be associated to other bacterial taxa, rather than *S. symbiotica*. We have analysed representatives from the different genera of the Lachninae with both FISH and PCR, and have surprisingly corroborated that many posses distinct co-obligate endosymbionts. While for most it is *S. symbiotica*, some members have lost this and replaced it by at least two different bacterial taxa. Therefore, this aphid subfamily could stand as a model for symbiont replacement and settlement, and through further genomic investigation, it is expected to shed light on the settling-down processes and the timing of the different colonization and genome reduction events.

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Symbionts protecting against multiple natural enemies

AILSA H. C. MC LEAN, BENJAMIN J. PARKER, JAN HRCEK, H. CHARLES J. GODFRAY

University of Oxford, UK

Protective symbionts can allow an otherwise vulnerable organism to acquire defence against a natural enemy. Aphids are one such example, with at least seven species of facultative bacterial symbiont known to provide protection against pathogens or parasitoids. Most individual aphids host one or two of these species, but the degree of protection conferred varies considerably between symbiont strains and between different species of natural enemy. For example, we have observed that protection against parasitoids by the symbiont *Hamiltonella defensa* is asymmetric with respect to different parasitoid wasp species. We hypothesized that asymmetric protection could affect interactions between the two parasitoids when in competition for aphid hosts. When the wasps are under intrinsic competition (two wasp eggs in the same host), we found that the outcome of the interaction is highly dependent on the presence or absence of a protective symbiont strain. The symbiont is therefore influencing not only the bipartite interaction between host and attacker, but also the interactions occurring between species at the trophic level above the host. Our results demonstrate the need to consider multiple players at multiple trophic levels, rather than a simple one symbiont/one host/one enemy system, in order to understand the far-reaching consequences of symbiosis for ecosystem structure and interactions.

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Mutualistic ants as an indirect defence against leaf pathogens

MARCIA GONZÁLEZ-TEUBER

Departamento de Biología, Universidad de La Serena, Casilla 554, La Serena, Chile.

Mutualistic ants are commonly considered as an efficient indirect defence against herbivores. Nevertheless, their indirect protective role against plant pathogens has been scarcely investigated. The protective role against leaf pathogens of two different ant partners, a mutualistic (*Pseudomyrmex ferrugineus*) and a parasitic (*P. gracilis*) ant, on the host plant *Acacia hindsii* (Fabaceae) was investigated. The epiphytic bacterial community on leaves was evaluated in the presence and absence of both ant partners by cultivation and by 454 pyrosequencing of the 16S rRNA gene. Pathogen-inflicted leaf damage, epiphytic bacterial abundance (colony-forming units) and the number of operational taxonomic units (OTUs) were significantly higher in plants inhabited by parasitic ants than in plants inhabited by mutualistic ants. Unifrac unweighted and weighted principal component analyses showed that the bacterial community composition on leaves changed significantly when mutualistic ants were removed from plants or when plants were inhabited by parasitic ants. Ant-associated bacteria would contribute to the protective role against pathogens. Some bacterial genera, widely known for their production of antibacterial substances, were found to live in ant legs. The results suggest that the indirect defence of mutualistic ants also covers the protection from bacterial plant pathogens. Moreover, the findings highlight the importance of considering ant bacteria as additional partners in ant–plant defensive mutualisms, which can contribute significantly to ant-mediated protection from plant pathogens.

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The good, the bad, and their regulation: Symbiosis, parasitism, and immune response in an insect gut

THOMAS ONCHURU[1], HASSAN SALEM[1], EUGEN BAUER[2], MARTIN KALTENPOTH[1]

[1] *Max Planck Institute for Chemical Ecology*
[2] *University Luxembourg*

The evolutionary and ecological success of many insect species is linked to their mutualistic associations with bacteria. These bacteria play important roles of nutrition and defense. Despite the well-established symbiont functions to hosts, little is known about their regulation and interaction with other intestinal organisms such as parasites. African cotton stainer bugs (*D. fasciatus*) have a relatively simple but well conserved gut microbiota, of which two *Actinobacteria* (*Coriobacterium glomerans* and *Gordonibacter* sp.) are essential in B-vitamin supplementation. In addition, *D. fasciatus* is a host to *Leptomonas pyrrhocoris*, an insect trypanosomatid parasite that is persistently present in natural and laboratory populations. In this study, we use molecular techniques to understand how the host maintains a homeostatic state of essential symbionts, while at the same time controlling parasite populations. Using comparative transcriptomic and qPCR analyses, we show differential expression of c-type lysozyme, defensin, pyrrhocoricin and hemiptericin AMPs between symbiotic and aposymbiotic host guts. Suppressing expression of these AMPs by RNAi and subsequent monitoring of changes in gut microbiota composition may conclusively elucidate their exact functions. To understand how parasites persist in *D. fasciatus* populations, we hypothesized that due to their co-localization with the symbionts, parasites may take advantage of the well-established symbiont transmission mechanism to infect subsequent generations. Indeed, experimental disruption of symbiont transmission by surface sterilization of eggs followed by diagnostic and qPCR analyses of hatchlings show that this disruption not only results in apo-symbiotic but also apo-parasitic hosts. This demonstrates how parasites hijack host-symbiont interactions to get vertically transmitted. Investigations into host-parasite interaction and putative role of symbiont in host defense are necessary to comprehensively understand multi-partite interactions in *D. fasciatus*. With these findings, we show that simple model systems such as the cotton stainer can be used to understand host-symbiont-parasite interactions in multi-organisms.

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Using three-way interactions between plants, microbes and arthropods to enhance crop protection and production

MARIA J.POZO

Consejo Superior de Investigaciones Científicas, Spain

Crop plants interact with both arthropods and microorganisms, including pests that reduce yields (in Europe up to 20% annually) and mutualists that promote yield. Direct and indirect interactions between microorganisms and arthropods on crops can strongly modify their impacts on yield. For instance, herbivores and pathogens can facilitate each other, causing additional yield loss. On the other hand, beneficial microorganisms can induce defenses that protect plants against herbivores. There is thus potential to enhance crop production and reduce pesticide use if we can better predict and manage Crop-Arthropod-Microorganism (CAMo) interactions to our advantage. Currently, knowledge of CAMo interactions is limited due to historical separation of the involved research fields. This COST Action will therefore combine existing expertise on CAMo interactions in Europe, from basic and strategic research to agri-R&D companies, and form an interdisciplinary platform and incubator for research on mechanisms, impacts and utilization of CAMo interactions on crop yield. The COST Action will also strengthen the careers of both female and young researchers, connect the newest research in the field with its applied use, and develop new monitoring and management support systems and CAMo-based applications.

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Starvation response in the plastid-bearing sea slug *Elysia viridis*

CESSA RAUCH[1], GREGOR CHRISTA[1,2], JOÃO SERÔDIO[2], SÓNIA CRUZ[2], SVEN B. GOULD[1]

[1] *Institute for Molecular Evolution; Heinrich-Heine-University, Düsseldorf, Germany.*
[2] *CESAM - Centro de Estudos do Ambiente e do Mar, Universtiy of Aveiro, Aveiro, Portugal*

Elysia viridis (Gastropoda, Sacoglossa) is one of the few known sea slug species that can survive starvation long-term in the presence of stolen plastids (kleptoplasts). Recently, a detailed study on two related sister taxa, *Elysia timida* and *Elysia cornigera*, demonstrated that these two responded very differently to starvation although both fed on the same food alga, *Acetabularia*. In this respect *E. viridis* is special, because it can feed on several different algal species and its starvation period might dependent on the food source. This is a unique system within the Sacoglossa and allows analyzing the effect of the food source on the response to starvation. We collected *E. viridis* from the costal shores of Zeeland, Netherlands and from Praia de Barra, Portugal and fed them in the laboratory solely on *Codium* or on *Bryopsis*. Slugs were then separated into different groups (dark, light, chemically blocked photosynthesis and a control starvation group) for analysis during starvation. For specimens fed on either of the two food sources we the PSII quantum yield was measured. In general, specimens fed on *Bryopsis* lost their functional plastids quicker and die earlier, but the importance of photosynthesis remains questionable. Furthermore, specimens fed on *Codium* showed lower levels of ROS evolution, which is reminiscent of what was observed for another long-term retention species. It suggests that the algal source from which plastids are sequestered has a direct impact on the survival rate of *Elysia viridis*. Our results, once more, provide evidence that the right plastid source is as essential as the adaption of the slug biology to endure starvation.

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Choose your partner carefully: variation in aphid phenotypes when multiple bacterial symbionts coexist

MELANIE SMEE, SALLY RAINES, JULIA FERRARI

University of York, UK.

In nature, it is common for insects to harbour multiple symbionts which may themselves have conflicting or agreeing interests. Currently, little is known about the interactions between multiple species of facultative symbionts occurring within an individual aphid. With multiple partners within one host, there may be selection for either cooperation or competition, and it may not always be beneficial to host more than one symbiont. In the pea aphid, *Acyrthosiphon pisum*, bacterial symbionts have a wide range of ecological effects, from protection from natural enemies to host plant utilisation. It has recently become clear that different strains of the same facultative symbiont species vary in their effects. Multiple symbiont species that coexist within the same host therefore allow us to study how a community evolves in response to selection on multiple levels. We used *A. pisum* individuals infected both singly and doubly with strains of *Hamiltonella defensa* and the X-type symbiont, to investigate how combinations of two common symbionts affected aphid phenotypes in different ecological situations. We found that the outcome of double infections is strongly dependent on the combination of individual strains of the two symbionts present and on the ecological context, highlighting the importance of genotype x genotype x environment interactions.

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Molecular features of the receptor for activated C kinase from *Symbiodinium microadriaticum ssp. microadriaticum*, SmicRACK1

TANIA ISLAS-FLORES[1], ESMERALDA PÉREZ-CERVANTES[1], JESSICA NAVA-GALEANA[1], GABRIEL GUILLÉN[2], MARCO A. VILLANUEVA[1]

[1] *Unidad Académica de Sistemas Arrecifales, Instituto de Ciencias del Mar yLimnología-UNAM, PO BOX 1153, Cancún, Quintana Roo 77500, México.*
[2] *Depto. de Biología Molecular de Plantas, Instituto de Biotecnología-UNAM, PO BOX 510-3, Cuernavaca, Morelos, México.*

Symbiotic dinoflagellates of the genus *Symbiodinium* are poorly studied in terms of their signal-transduction pathways, which may reveal important functional clues towards their physiology and symbiotic associations. We PCR-amplified, cloned and sequenced the RACK1 homolog from these cells (SmicRACK1) and through further inverse PCR amplification and sequencing of genomic DNA, we found at least two genes which we termed SmicRACK1A and SmicRACK1B. These isogenes varied in only two bases (C/T and T/C) which represented two synonimous substitutions since they encoded identical amino acid sequences. Both open reading frames were in tandem separated by an intergenic sequence of 359 nucleotides within which, light-dependent regulatory regions were detected. Both ORF's displayed 942 bp, which translated to 313 amino acids with the characteristic seven WD40 repeats and three PKC binding sites. The theoretical molecular size and pI of the protein were 34,200 and 5.9, respectively. SmicRACK1 showed more divergency at the amino acid level, than at the nucleotide level. Maximum identities observed at amino acid and nucleotide levels, respectively, were 80 and 85% with *Heterocapsa triquetra*, 68 and 76% with *Toxoplasma gondii*, 68 and 69% with *Perkinsus marinus*, and 63 and 72% with *Chlamydomonas reinhardtii*. Phylogenetic relationships revealed a cluster of related sequences from the marine dinoflagellates *H. triquetra, Karenia brevis* and *Alexandrium tamarense* and a slight more distance, from *Oxhyrris marina*. Its closest independent cluster grouped sequences from the protozoan parasites *Toxoplasma gondii* and *Neospora caninum*. Our results illustrate the genomic organization of SmicRACK1 and its molecular features suggest its classical role as a scaffolding protein within light-regulated signal-transduction pathways. We are currently studying its possible role in cnidarian-dinoflagellate symbiotic interactions.

Acknowledgements: This work was supported by grants PAPIIT IN-210514 from DGAPA-UNAM and 175951 from the National Council for Science and Technology (CONACYT, Mexico).

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Do symbiotic gut bacteria influence interactions between ants and lycaenid butterflies?

MELISSA R.L. WHITAKER[1,2], MARTIN KALTENPOTH[1], NAOMI E. PIERCE[2]

[1] *Max Planck Institute for Chemical Ecology*
[2] *Harvard University, Museum of Comparative Zoology*

It is well documented that the symbiotic gut microbes of insects can influence the fitness of their hosts via effects on metabolism, behavior, defense, reproduction, nutrition, and immunity. Microbes may also influence their hosts' associations with other organisms, though little research has explored how symbiotic microbes affect insects' interactions with free-living species. To explore this issue I will present results from a microbial community analysis that investigates the taxonomic, functional and evolutionary dimensions of the gut microbiomes of lycaenid butterflies. The majority of lycaenid species engage in highly specialized interactions with ants during their larval (and often pupal) stages, requiring considerable physiological, behavioral, and ecological adaptations related to attracting and maintaining ants' protective care. Most associations between lycaenids and ants are mutualistic, though parasitic lifestyles have evolved independently in multiple lycaenid lineages, wherein larvae switch from herbivory to entomophagy and feed on their host ants or ant-tended insects. Lycaenid butterflies therefore represent an excellent system for exploring the potential for symbiotic microbes to influence host fitness in previously undocumented ways, namely by mediating their interspecific interactions with other organisms. I collected lycaenid larvae belonging to 32 species and spanning the full range of dietary diversity from Africa, the USA, and southeast Asia, and characterized their gut bacterial communities using 16s amplicon sequencing. The resulting data set represents an extensive single-family survey of insect-associated gut bacteria, allowing for a comparison of the relative contributions of phylogeny and diet to differences in lycaenids' microbial communities. I identified bacterial taxa that are differentially abundant in mutualistic versus parasitic lycaenids, characterized the putative functional capabilities of the bacterial communities associated with different lycaenid groups, and discuss the roles of both microscopic and macroscopic players in the context of multipartite species interactions.

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Session Chairs: Russell J. Rodriguez[1], Stanley Freeman[2]
Chair affiliations: [1]Adaptive Symbiotic Technologies, USA
[2]The Volcani Center, Israel

Symbiotic lifestyle switching

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Session: Symbiotic lifestyle switching
Presentation type: Oral
Name, Contact: Anna Zaidman-Rémy, anna.zaidman@insa-lyon.fr

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How do insects optimize their symbiotic investment? Endosymbiont control and load adjustment to insect physiological needs

AURÉLIEN VIGNERON, FLORENT MASSON, AGNÈS VALLIER, SÉVERINE BALMAND, MARJOLAINE REY, CAROLE VINCENT-MONÉGAT, EMRE AKSOY, ETIENNE AUBAILLY-GIRAUD, ANNA ZAIDMAN-RÉMY, AND ABDELAZIZ HEDDI

Laboratoire Biologie Fonctionnelle, Insectes et Interactions, INSA de Lyon.

Insects living on nutritionally poor ecological niches highly rely intracellular symbiotic bacteria (endosymbionts) that supply them with metabolic components lacking in their diet. How insects balance costs and benefits of hosting endosymbionts, and whether and how they adjust symbiont load to their physiological needs and environmental conditions, remains largely unexplored. By examining the association between cereal weevils *Sitophilus* spp. and their endosymbiont *S. pierantonius*, we unraveled an extreme modulation of endosymbiont load during insect development. Weevil endosymbionts first intensively multiply in young adults, supporting a massive need for Tyr and Phe amino acids, which are the precursors of the DOPA molecule required for the completion of their exoskeleton – the cuticle that preserves the insects from desiccation, shocks and pathogens. Comparison between weevil species from different ecological niches further suggests a modulation of endosymbiont load according to environmental and nutritional conditions. Once the cuticle is achieved, DOPA reaches high amounts in insects, which triggers endosymbiont elimination in a tissue-dependent manner that preserves germline-associated endosymbionts and thus their transmission to next generations. Endosymbiont elimination involves apoptosis and autophagy, which processes avoid inflammation and allow the recycling of the bacterial components. While mutualism significantly impacts organism life history traits, enhancing adaptive features such as the cuticle thickness, cooperative associations remain under the control of a fine-tuned integration of costs and benefits. The synchronization of a “somatic” breakdown of symbiosis with the achievement of a specific developmental trait minimizes insect investment in symbiosis while preserving transmission to the progeny. We currently are investigating in more details the symbiotic partners’ interactions that underlie this striking dynamics, and how it has been evolutionary shaped through the insect nutritional specialization on cereals.

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Symbiosis establishment in the rhizosphere: specific tasks for specific partners

FRANCISCO CERQUEIRA[1], MANUELA CAROLINO[1], PATRICIA CORREIA[1], KHALID AKDI[2], CRISTINA CRUZ[1], LUÍS CARVALHO[1]

[1] *CE3C, FCUL, Lisboa Portugal*
[2] *AMC-Trichodex, Sevilha, Spain*

The rhizosphere is a hotspot of biodiversity with intensive microbial networking. How is it assembled? Is it a structured "team" of microorganisms working together or a haphazard assemblage of microbes? To answer this question, we propose a mechanistic model for the plant–microbiome interactions in the rhizosphere, and evaluate the specificity of the actors involved in some key functions of the rhizospheric community assemblage, namely microbial recruitment and biofilm formation. Plants and mycorrhizal fungi may have a role in attracting microbes to the rhizosphere. To verify this, we studied the effectiveness of maize (*Zea mays* L.) roots colonized by distinct arbuscular mycorrhizal fungi (AMF) in recruiting *Azospirillum brasilense* and inducing biofilm formation. We tested if root exudates from maize plants colonized or not with different AMF species generate *A. brasilense* chemotaxis and stimulate biofilm formation, and if bacterial interspecific interactions influence *A. brasilense* biofilm formation. Results showed that *G. mosseae* and *G. claroideum* had noticeable effects on *A. brasilense* chemotaxis, mediated through the root exudates. However, there was not any influence of the AMF on biofilm formation. Biofilm formation by *A. brasilense* required the presence of bacteria. Of the soil bacterial strains tested, *Pseudomonas putida* X236 had a positive effect on biofilm formation. To achieve a symbiotic lifestyle, and promote plant growth, *A. brasilense* needs to form a biofilm. In addition, our study showed that its performance as a symbiotic partner depends on the co-partners: 1) specific AMF modulate the efficiency of the root exudates in microbial recruitment; and 2) specific bacteria promote biofilm formation. Together, the results favour the hypothesis that the rhizospheric community is a selection of microorganisms working in a team with specific functions under strict social control.

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Living together, behaving as one: rethinking mycorrhizal systems

ANA CORRÊA[1], NURIA FERROL[2], CRISTINA CRUZ[1]

[1] *Center for Ecology, Evolution and Environmental Changes, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal*
[2] *Departamento de Microbiología del Suelo y Sistemas Simbióticos, Estación Experimental del Zaidín, CSIC, Granada, Spain*

Mycorrhizae are widespread symbiotic associations formed between plants and fungi. The main benefit to the plant from mycorrhizae is generally considered to be improved nutrition, in exchange for which the plant provides the fungal partner with C. Mycorrhizae are generally considered to be mutualisms. However, increasing observations of negative and null responses of plant productivity to mycorrhization have led to the concept of mutualism-parasitism continuum of mycorrhizal function. This is a phytocentric concept, focused on the symbiosis effect on plant productivity. The basis for parasitism would be excessive C drain by the fungus. In this context, mycorrhizal symbioses are evaluated by comparison with the non-symbiotic plant. If plant productivity in unchanged, or if it decreases with the symbiosis, this is interpreted as it being a comensalism or a parasitism, respectively. This conclusion is not made based on the observation of parasitic mechanisms, rather it is based on comparisons with a different system (non-symbiotic plant). However, there is increasing evidence that mycorrhizal partners exchange excess resources, i.e., that they only trade nutrients they have in excess and cannot use for their own growth, and that negative effects on plant growth are not always associated with the C costs of the symbiosis. This disputes some basic assumptions of the mutualism-parasitism continuum in mycorrhizae. We contest the notion of a parasitism-mutualism continuum in mycorrhizal symbioses. We propose that rather than a functional continuum, there is a continuum of conditions over which a plant in a mycorrhizal system will sometimes have an advantage when compared with a non-mycorrhizal plant, and other times not. Furthermore, we propose the rethinking of mycorrhizal associations as mycorrhizal systems responding to the environment as one, rather than mycorrhizae as a factor affecting plants.

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Symbiotic lifestyles between and among three fungal species with the ambrosia beetle *Euwallacea* nr. *fornicatus* and different host plants

STANLEY FREEMAN[1], MICHAL SHARON[1], MARCEL MAYMON[1], MALLY DORI-BACHASH[1], ALEX PROTASOV[2], OMER MARGALIT[2], KEERTHI MOHOTTI[3], AND ZVI MENDEL[2]

[1] *Dept. of Plant Pathology and Weed Research*
[2] *Dept. of Entomology, Agriculture Research Organization, The Volcani Center, Bet Dagan 50250, Israel;*
[3] *Tea Research Institute, Entomology and Nematology Division, St. Coombs, Talawakelle 22100, Sri Lanka*

The ambrosia beetle, *Euwallacea nr. fornicatus* (Coleoptera: Scolytinae), is a new invasive species to Israel, first reported in 2009. It has become a severe pest of avocado, with c.a. 80% of the plantations in Israel being affected to date. Isolations from female beetle mandibular mycangia and brood galleries in the trees revealed the presence of three fungal symbiotic species: *Fusarium euwallaceae, Graphium euwallaceum sp. nov.* and *Acremonium roseum* sp. nov. (the latter two fungi recently described by Lynch *et al.* 2015). Both *G. euwallaceum* and *A. roseum* mainly occur on callow adults, whereas isolations from mature adult beetles revealed *F. euwallaceae*, almost exclusively. The beetle is attracted to the sapwood of 48 tree species in 25 botanical families in Israel. In most of these tree species the penetration attempts resulted with minimal or no apparent fungal colonization; and in both instances the beetle did not reproduce. Successful colonization of the 10 tree species, which resulted with brood development, also coincided with inoculation of the xylem galleries with the three symbiotic fungi. Larvae feed and complete their development on *F. euwallaceae* and *G. euwallaceum* but not on *A. roseum*, nor on non-specific *Fusarium* spp. that served as controls. Artificial inoculation with *F. euwallaceae* and *G. euwallaceum* of xylem disks from different plant host species were conducted in plates with the beetle. Even non-hosts such as persimmon and brachychiton resulted in successful larvae development, mainly with *F. euwallaceae*, while both fungi allowed adult beetle disk colonization. Only *F. euwallaceae* (mainly from the adults) and *G. euwallaceum* (mainly from the larvae) were isolated from stomachs of the tested beetles. The symbiotic interactions of *F. euwallaceae, G. euwallaceum* and *A. roseum* with the larvae and beetle, and their pathogenic role in avocado, will be discussed.

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Session: Symbiotic lifestyle switching
Presentation type: Keynote
Name, Contact: Regina S. Redman, RSRedman@AdSymTeh.com

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The role of genetic and environmental landscapes on plant-fungal symbiosis

REGINA S. REDMAN[1], RUSTY J RODRIGUEZ[1,2]

[1] *Adaptive Symbiotic Technologies (AST), Seattle WA USA*
[2] *University of Washington, Department of Biology, Seattle WA USA*

The outcome of plant-fungal associations can range from parasitism to mutualism depending upon the genetic landscape of the symbiotic partners. We began our studies by asking "Are pathogens always pathogens, or can they express alternate fungal lifestyles depending upon the plants with which they are associated?" We found that different species of Colletotrichum known as pathogens had the ability to express alternate lifestyles and become endophytic mutualists on plants thought to be non-hosts. Expressing mutualistic lifestyles, the pathogens were able to asymptomatically colonize all parts of the plant except for the seed embryo and could confer positive symbiotic benefits (disease resistance, drought tolerance, growth and yield enhancement). All plants in natural ecosystems are thought to have associations with fungal endophytes that can have profound impacts on the health and survival of plants confronted with abiotic stresses. Analysis of native plants growing across soil temperature and salinity gradients revealed that the level of stress determines the outcome of symbiotic relationships. Across these stress gradients plants changed endophyte associations to achieve optimal fitness. Endophytes also changed plant host in adjacent microhabitats imposing different stresses. We define this phenomenon as "Symbiotic Modulation" and hypothesize that the environmental gradient is playing a driving role in the outcome of symbiotic associations. Moreover, fungi isolated from plants growing across these environmental stress gradients, conferred levels of stress tolerance similar to the microhabitat from which plants were isolated. Endophytes from along the stress gradients conferred similar levels of stress tolerance to agricultural crop plants. We present a strategy based on Symbiotic Modulation to mitigate impacts of climate change on plant communities.

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Characterization of *Sodalis glossinidius* heme iron acquisition and homeostasis genes

LAURA J. RUNYEN-JANECKY[1], BECKY CHEN[1], THOMAS ARENA[1], BRIAN WEISS[2], AND SERAP AKSOY[3]

[1] *Biology Department, University of Richmond, Richmond, Virginia 23173, United States*

Sodalis glossinidius, a facultative intracellular symbiont of the hematophagous tsetse fly (*Glossina* sp.), resides in a wide variety of tsetse tissues and, thus, may encounter environments that differ dramatically in iron content. Despite significant genome erosion, *Sodalis* has maintained genes for several iron homeostasis systems. The *Sodalis* hemR gene encodes a membrane-localized protein that binds heme and has homology to heme iron transport proteins in other bacteria. Expression of the *Sodalis* hemR gene in an *Escherichia coli* strain unable to use heme as an iron source stimulated growth in the presence of heme or hemoglobin as the sole iron source. However, the *Sodalis* hemR gene could not complement a heme auxotrophy in *E. coli* suggesting that only the iron, and not the entire heme molecule, is transported into the cytoplasm. Adjacent to hemR on the chromosome is the hemS gene, which encodes a protein with homology to cytoplasmic proteins that can extract iron from heme, sequester heme, and/or transfer heme to heme degradation proteins. Like hemR, the hemS gene was expressed by *Sodalis* in the tsetse fly; however, expression of hemS was not repressed by iron. *Sodalis* hemR mutant strains were defective in their ability to colonize the gut of tsetse flies that lacked endogenous symbionts, and current work is examining the phenotype of *Sodalis* hemS mutant strains in these tsetse flies. Maintenance and expression of bacterial iron acquisition and regulation genes when *Sodalis* is in the tsetse fly suggests that iron homeostasis maybe important for the maintenance of the symbiotic relationship.

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Active invasion of bacteria into living fungal cells

ZERRIN ÜZÜM[1], NADINE MÖBIUS[1], JAN DIJKSTERHUIS[2], GERALD LACKNER[1], CHRISTIAN HERTWECK[1]

[1] *Leibniz Institute for Natural Product Research and Infection Biology (HKI), Dept. Biomolecular Chemistry, Jena, Germany.*
[2] *CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.*

The rice seedling blight fungus *Rhizopus microsporus* and its endosymbiont *Burkholderia rhizoxinica* form an unusual, highly specific alliance to produce the highly potent antimitotic phytotoxin rhizoxin [1]. Gain of resistance to rhizoxin by a mutation on fungal genome initiated the co-evolution of the mutual counterparts and switch of parasitism to mutualism [2]. In this obligate endosymbiotic interaction, the fungus fully depends on the bacterial presence to form vegetative spores, and the bacteria are transmitted vertically via fungal spores. In contrast to many known endosymbiotic fungal-bacterial interactions, both *R. microsporus* and *B. rhizoxinica* can be maintained in axenic cultures. Re-establishment of the endosymbiosis is possible by co-culturing of the sterile counterparts. Thus, it represents an ideal model system to study endosymbiotic interactions [3]. Yet, it's remained a riddle how bacteria invade the fungal cells. We aimed at understanding the molecular mechanisms of colonisation and the maintenance of the endosymbiotic interaction. In silico predictions of potential bacterial symbiosis factors and functional analyses revealed that a type 2 secretion system (T2SS) of the *B. rhizoxinica* is required for the establishment of the endosymbiosis. Comparative proteome analyses showed that the T2SS releases bacterial chitinolytic enzymes (chitinase, chitosanase) and chitin-binding proteins. By means of targeted gene knock-outs, sporulation assays and microscopic investigations we found that the chitinase is essential for *B. rhizoxinica* to enter the hyphae. Unprecedented snapshots of the traceless bacterial intrusion were obtained using cryo-electron-microscopy. Beyond unveiling the pivotal role of chitinolytic enzymes in the active invasion of a fungus by bacteria, these findings grant unprecedented insights into the fungal cell wall penetration and symbiosis formation [4].

[1] Partida-Martinez & Hertweck (2005): *Nature 437*, 884-888.
[2] Schmitt, *et al.* (2008): *ISME J 2* 632-641.
[3] Partida-Martinez, *et al.* (2007): *Curr. Biol. 17* 773-777.
[4] Moebius & Uzum, *et al.* (2014): *eLife 3* e03007.

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The proposed role of virulence-suppressive compounds in maintenance of latency in cranberry fruit rot disease and its relevance to endophytism

MARIUSZ TADYCH, STEPHANIE FONG, YIFEI WANG, MARSHALL BERGEN, JENNIFER JOHNSON-CICALESE, NICHOLI VORSA, JAMES WHITE

Department of Plant Biology and Pathology, Rutgers University, New Brunswick, New Jersey USA

Symbiotic lifestyle switching is evident in infection progression of fruit rot in cranberries. Fruit rot is caused by a complex of fungi (including species of *Colletotrichum, Coleophoma, Phyllosticta* and *Physalospora*) that infect cranberry ovaries early in development, remaining latent during fruit expansion. After fruit becomes mature fruit rot fungi begin to cause rot in fruit. In this research we sought to answer the following questions: 1.) Are there compounds in cranberry fruit that stimulate or inhibit growth or reactive oxygen (H_2O_2) production? 2.) Do levels of those compounds vary as fruit develops toward maturity and rot is initiated? We began the study by screening compounds known to occur in cranberry fruit for their capacity to inhibit growth or reactive oxygen secretion by several species of cranberry rot fungi. We identified quinic and benzoic acids as active in reducing growth and suppressing reactive oxygen secretion while other compounds did not inhibit growth and reactive oxygen production. We further examined levels of quinic and benzoic acids during fruit development. Results of our studies suggest that during the latent phase virulence of fungi is suppressed by quinic and benzoic acids that function as oxidase inhibitors to prevent production of reactive oxygen that initiates the hypersensitivity (rot) process. Only after levels of quinic acid fall, and before benzoic acid levels rise in fruit, do fungi begin to initiate rot disease. In the cranberry fruit rot system the host appears to have regulatory control of virulence activities of the pathogens, maintaining them in a non-virulent phase until fruit development is complete. This is significant because comparable reactive oxygen-suppression mechanisms could function in other plants where pathogenic microbes live as asymptomatic endophytes. Reactive oxygen- or oxidative burst-suppressive compounds produced either by the microbes or the hosts could play regulatory roles in maintaining non-pathogenic states.

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Session Chairs: Katarzyna Turnau[1], Cristina Prandi[2]
Chair affiliations: [1]Jagiellonian University, Poland
[2]Università di Torino, Italy

Symbiotic microbes - new frontiers in applied biotechnology

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Exploring the supply and recycling of nutrients of *Ramalina farinacea*-associated culturable bacteria: contribution to the lichen symbiosis and biotechnology

ÁNGELA FIGAS-SEGURA[1,2], EVA BARRENO[2] AND ELENA G. BIOSCA[1]

[1] *Departamento de Microbiología y Ecología, Facultad de Ciencias Biológicas, Universitat de València.*
[2] *Instituto Cavanilles de Biodiversidad y Biologia Evolutiva, ICBIBE,Dpto.Botánica,Fac. Biológicas.1,2.C/Doctor Moliner 50,46100, Burjasot,Valencia*

Lichens are complex symbiotic associations of a mycobiont and one or more photobionts, but other partners such as non-photosynthetic bacteria can be present in lichen thalli. The characterization of the diversity and physiology of these bacteria is crucial to understand microbial interactions in the lichen symbiogenesis, but also to explore their biotechnological potential.In this work, *Ramalina farinacea* (L.) Ach. thalli growing in *Pinus canariensis* forests, under different bioclimatic conditions, were collected in Tenerife (Canary Islands). A large collection of ectolichenic and endolichenic culturable bacteria, isolated from these thalli by using a novel protocol, was characterized by testing several activities related with nitrogen, phosphorous and iron supply, as well as diverse hydrolytic activities potentially involved in nutrient recycling within lichen thalli. Selected strains were identified by partial 16S rRNA gene sequencing. The results revealed nitrogen fixation, phosphate-solubilizing and siderophore-producing capabilities in 92%, 54% and 84% of the strains, respectively. These bacterial activities may provide limiting nutrients to the lichen thallus, being also of interest in agricultural biotechnology. Further, amylases, lipases, proteases and nucleases were detected in 47%, 64%, 34% and 16% of the strains, respectively, and cellulases, pectinases, xylanases and chitinases in about 25% of them. Such activities could contribute to nutrient recycling in senescent parts of lichen thalli. This hydrolytic potential could also be exploited in plant residues bioremediation, biocontrol of fungal pathogens and food and/or pharmaceutical industries. Molecular identification of selected bacterial strains revealed the association of *R. farinacea* with different taxa, some of them still little studied. Conclusions: Bacterial communities associated to *Ramalina farinacea* could contribute to the lichen symbiosis through the provision and the recycling of nutrients. They should also be explored as a source of new microorganisms with different biotechnological potentials.

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Why the recognition of the identity of arbuscular mycorrhizal fungi (Glomeromycota) is essential to know and practically use their symbiotic lifestyle

JANUSZ BŁASZKOWSKI

Department of Ecology, Protection and Shaping of Environment, West Pomeranian University of Technology in Szczecin, Słowackiego 17, PL-71434 Szczecin, Poland

Arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota have a worldwide distribution and are associated with at least 70% of vascular land plant species and those living under water. Molecular studies and fossil evidence suggest that the fungi evolved concurrently with land plants and played a crucial role in colonization of the land by plants. Currently we know that AMF also increase the supply of plants with nutrients, render them less sensitive to different abio- and biotic disease factors and influence soil structure and the composition and functioning of entire plant ecosystems and other associated microorganisms. However, there is an increasing body of evidence indicating that the effects of the symbiosis highly depend on the identity of AMF coexisting with plants and that different external factors, especially human impacts, may significantly shape the effects. Thus managing these changes in the expected direction have to be based on unambiguous identification of the main initiators of the changes, i.e. AMF. Therefore the aims of my presentation will be to show the fundamental principles and difficulties of morphological and molecular identification of AMF, as well as the importance of knowledge on the intraspecific relationships in the fungi to fruitfully modify their fascinating symbiotic lifestyle. I will present and discuss among others the following issues: (i) the diversity of phenotypic and histochemical features of spores and mycorrhizal structures and their significance and use in identification and classification of AMF, (ii) the reasons of difficulties or impossibilities of determining relationships between AMF based on morphology only and (iii) advantages and imperfections of methods of molecular recognition of AMF.

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The microbiome of the Antarctic oligochaeta *Grania* sp.: occurrence of hydrolytic enzyme-producing microorganisms

LORENA HERRERA[1], JUAN JOSÉ MARIZCURRENA[1], RODRIGO PONCE DE LEÓN[1], SUSANA CASTRO-SOWINSKI[1]

[1] *Sección Bioquímica y Biología Molecular,*
[2] *Sección Zoología de Invertebrados, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay.*

Grania sp. is an oligochaeta found in aquatic and semiterrestrial environments. They inhabit the coast of the Maxwell bay (King George Island, Fildes peninsula, Antarctica) and are responsible of the degradation of red and brown algae, which invade the shore, probably using their chemical components as carbon and energy source. The aim of this work was to analyze the gut microbiome of this oligochaeta, focusing on the isolation and identification of culturable hydrolytic-enzyme producing microorganisms. The oligochaetas were washed with sterile distilled water and the gut was aseptically removed. The guts were suspended in sterile water and mechanically crushed. Then, the solution was spread on differential media for the isolation of proteolytic (skim milk), lipolytic (olive oil), cellulolytic (carboxymethyl cellulose, CMC) and amylolytic (soluble amylose) and incubated at 10ºC. Microorganisms that were able to hydrolyze milk proteins, CMC and amylose, and to produce a pit on agar (agarolytic bacteria) were isolated. Microorganisms were classified as bacteria or yeast after Gram-staining and microscopy observation. Based on extracellular enzyme production and phenotypic morphology of colonies, ten bacteria were chosen for further analysis. It was found that several isolates produced many extracellular hydrolytic enzymes. Bacteria were identified by sequencing of the 16S rDNA gene and analysis in the ribosomal data base. Results showed that they are members of the bacterial genera *Flavobacterium, Psychrobacter, Pseudomonas* and *Rahnella/Serratia*, some of them showing high identity to bacteria isolated from Antarctic cod´s and salmon's guts, and algae. Yeasts have not yet been identified. The results so far suggest that the Antarctic *Grania* sp. possess a gut microbiome that produce hydrolytic enzymes (proteases, cellulases, amylases and agarases) probably involved in nutrition. We do not exclude the possibility of cross-contamination with microorganisms present in the surface of *Grania* sp. Future work will deal with yeast identification and enzyme characterization.

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Draft genome of the plant-growth promoting and Cr(VI)-reducing bacterium *Delftia* sp. JD2

MARIA MOREL[1], ANDRES IRIARTE[2], EUGENIO JARA[3], HÉCTOR MUSTO[3], SUSANA CASTRO-SOWINSKI[1,4]

[1] *Microbiología Molecular, IIBCE, Montevideo, Uruguay.*
[2] *Bioquimica y Genómica Microbiana, IIBCE.*
[3] *Organización y Evolución del Genoma, Facultad de Ciencias, Udela R.*
[4] *Bioquímica y Biología Molecular, Facultad de Ciencias, Udela R.*

Delftia sp. JD2 is an auxin/siderophore plant-growth-promoting bacterium that promotes growth and nodulation of legumes when co-inoculated with rhizobial strains. Interestingly, JD2 reduces Cr(VI) to Cr(III) and shows multi-resistance to several heavy-metals and antibiotics. The aims of this work were: 1) to perform a comparative genomic analysis using the draft genome of JD2 and, 2) to analyze the molecular elements involved in the JD2 symbiotic phenotype, such as auxin- and siderophore-production, and including the study of the molecular elements involved in heavy-metal and antibiotic resistances. The genome of JD2 was sequenced and automatically annotated. The occurrence and genomic organization of pathways involved in auxin and siderophore production, polyhydroxy-alkanoates production and degradation, chromate and antibiotic resistances, among others, were analyzed. Results from comparative genomic analysis and phylogeny will be shown during the meeting. JD2 possess a 6.76Mb genome with 66.4% GC content and 98% identity with others *Delftia* annotated genomes. JD2 has numerous genes related to tryptophan synthesis and degradation, indol 3-acetic acid biosynthesis, siderophores production and secretion and TonB dependent transporters with various substrate specificities. Consistent with its environmental adaptability, JD2 contains a large number of genes involved in the uptake, transformation and efflux of several toxic compounds. Syntenic genes related to chromate resistance (chr), flanked by mobile elements, were identified. Interestingly, the phylogenetic analysis suggests that the chr cluster of others *Delftia* strains was lost during evolution. The results provide insights into the potential use of JD2 in heavy metal bio-remediation programs and biofertilization technologies as an interestingly partner during the symbiotic association between rhizobia and legumes, as suggested by Morel *et al* (2011, 2015) and Ubalde *et al* (2012).

Morel *et al.* (2011): Arch Microbiol 193: 63-68
Ubalde *et al.* (2012): Curr Microbiol 64 :597-603
Morel *et al.* (2015): MPMI 28 : 134-142.

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Functional characterization of the TGFbeta pathway during the onset of symbiosis in a scleractinian model

OLIVIER DETOURNAY[1], JÉRÉMY BERTHELIER[1], ANGELA POOLE[2], CHRISTY SCHNITZLER[3], ELISHA WOOD-CHARLSON[4], VIRGINIA WEIS[2]

[1]*Coral Guardian*
[2]*Oregon State University*
[3]*NIH*
[4]*University of Hawaii*

Considering their ecological and economic value, coral reefs are one of the most important ecosystems for humankind. They are constituted by scleractinian which harbor photosynthetic dinoflagellate endosymbionts from the genus *Symbiodinium*. However, various environmental stressors, most notably elevated seawater temperature caused by global warming, can cause a breakdown in the partnership resulting in loss of symbionts from host tissues. This phenomenon, known as coral bleaching, results in greatly reduced host fitness and can lead to reef destruction. Our previous study performed in *Aiptasia pallida*, a symbiotic anemone, determined that the dinoflagellate symbionts promote host tolerance through the activation of the Transforming growth factor beta (TGFb) pathway. A defect in the activation of this pathway due to heat-stress or bacterial infection resulted in immune overstimulation and a failure of the symbionts to successfully colonize the host. The goal of this research was to characterize the function of the TGFb pathway in the onset of symbiosis between the planula larva of the solitary *Hawaiian scleractinian* coral *Fungia scutaria* and its dinoflagellate symbiont *Symbiodinium* sp. type C1f. We show evidence that (1) the TGFb transcript is expressed during the onset of symbiosis, (2) the inactivation of the TGFbeta pathway decreases the infection rate success with C1f dinoflagellate, and is (3) associated with the induction of nitric oxide secretion in the host. This work highlights the importance of immune tolerogenic pathways in mutualistic interactions and paves the way to a better understanding of how innate immune mechanisms are affected by environmental changes. Immunity in corals is almost completely unexplored to date and therefore this work is be of great interest, not only to those studying coral-dinoflagellate symbiosis but also to those in the fast-expanding field of coral disease.

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Presentation type: Oral
Name, Contact: Genre Andrea, andrea.genre@unito.it

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Presymbiotic signaling in arbuscular mycorrhizas

GIULIA RUSSO, MARCO CHIAPELLO, MARA NOVERO, PAOLA BONFANTE, ANDREA GENRE

Università di Torino - Department of Life Sciences and Systems Biology

The molecular signals present in arbuscular mycorrhizal (AM) germinated spore exudates are responsible for activating a signal transduction pathway that includes nuclear Ca_2^+ spiking in the host root epidermis.By using root organ cultures (ROCs) expressing a nuclear-localized cameleon reporter we developed a bioassay to detect AM-associated Ca_2^+ spiking responses and characterize bio-active molecules in fungal exudates. This approach has revealed that short-chain chitin oligomers (COs) can mimic AM fungal exudates in elicitating Ca_2^+ spiking, with maximum activity observed for CO 4 and CO 5. This spiking response is dependent on genes of the common SYM signalling pathway (DMI1/DMI2) but not on NFP, the putative *Sinorhizobium meliloti* Nod factor receptor. A major increase in the CO 4/5 concentration in fungal exudates is observed when *Rhizophagus irregularis* spores are germinated in the presence of the synthetic strigolactone analogue GR24. By comparison with COs, both sulphated and nonsulphated Myc lipochito-oligosaccharides (LCOs) are less efficient elicitors of Ca_2^+ spiking in *M. truncatula* ROCs.We propose that short-chain COs secreted by AM fungi are part of a molecular exchange with the host plant and that their perception in the epidermis leads to the activation of a SYM-dependent signalling pathway involved in the initial stages of fungal root colonization.A piece of software performing the automated analysis of Ca^{2+}+ spiking signals has been developed with the aim of characterizing global trends in large datasets. based on this analysis, variations in the regularity of the spiking pattern could be consistently recognized between spiking profiles triggered by COs, LCOs and Nod factors, suggesting that this could be part of the calcium signature encoded in such nuclear signals.

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Application of mycorrhiza symbiosis- present and future prospects

YORAM KAPULNIK

ARO, the Volcani Center, Bet Dagan, 50250 Israel

The application of Arbuscular Mycorrhiza Fungi (AMF) inoculum to the soil under sub-optimal growth conditions, has been proven to be effective in enhancement of plant growth and in increasing plant resistance against abiotic and biotic stress conditions. These features of AMF inoculum were demonstrated with several important crops under controlled or agronomical growth conditions. We have developed mycorrhiza inoculum that is cost effective for agriculture use. It is with high propagule number and long shelf life. This inoculum is being applied now to a variety of crops under commercial scale in different location worldwide and under different growth conditions. Here I will present some of the obtained results and discuss future prospective for mycorrhiza inoculum usage.

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Symbiotic microbes - new frontiers in applied biotechnology

KATARZYNA TURNAU

Jagiellonian University, Poland

The sustainability of natural resources has been severely threatened in the last centuries. In many cases irreversible changes in the environment make it impossible for it to be utilized for urbanization or agriculture. Out of the numerous aspects of environment degeneration the pronounced reduction in the biodiversity of symbiotic microorganisms clearly stands out. The importance of this phenomenon manifests itself on every level of biological organization, from individual organism to complex ecosystems.To prevent further degradation and for re-cultivation a multidisciplinary effort is needed. The development of systems biology based technologies for medicine, agriculture and industry seems inevitable to restrain the progressing environmental degeneration. Symbiotic microorganism, fungi and bacteria in particular can be used in re-cultivating sites degenerated. They have also been found to be very useful in medical biotechnology and disease control. During this session the latest accomplishment in the field of bioremediation and resotoration, symbiont based control of pests and disease, agricultural and industrial enhancement and ecology and medical implications of symbiosis research will be presented.

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Functional characterization of the bacterial community associated with a reproductive gland of the Hawaiian bobtail squid, *Euprymna scolopes*

ALLISON H KERWIN, SPENCER V NYHOLM

University of Connecticut

Many marine/aquatic organisms deposit their eggs in the environment where successful embryogenesis depends on minimizing biofouling and/or predation. The Hawaiian bobtail squid, *Euprymna scolopes*, harbors a diverse bacterial community within the accessory nidamental gland (ANG), a symbiotic organ associated with the female reproductive tract. Illumina sequencing of the 16S rRNA V4 gene region showed that this community is composed primarily of members of the Rhodobacteraceae and *Verrucomicrobia*, which together comprised on average 96% of the sequences recovered per sample (57% and 38% respectively, n = 11). The community composition of the ANG was similar to that of the egg jelly coat (JC, n=6), suggesting that bacteria from the ANG are deposited directly into the egg JC. Eggs treated with antibiotics over the course of embryogenesis developed a biofilm, primarily composed of the fungus *Fusarium solani*, which led to the death of the embryos (0% viability; n = 7 clutches). Transmission electron microscopy confirmed that both fungal hyphae and spores penetrated the jelly coat of antibiotic-treated eggs as far as the chorion. Taken together, these data suggest that the ANG/JC bacteria protect developing embryos from biofouling. To further investigate the role of the JC bacteria in egg protection, additional experiments are underway to test the susceptibility of different parts of the egg (capsule, jelly coat and embryo) to fungal infection. Future experiments will also investigate whether complementing with specific ANG bacterial isolates and compounds derived from these strains will prevent fungal infection of eggs. Understanding the function of the ANG bacterial consortium may lead to novel anti-fouling/antimicrobial compounds and lend insight into the mechanisms by which marine invertebrates protect their eggs in the environment.

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Potential practical implementation of strigolactones for plant interactions

HINANIT KOLTAI

Institute of Plant Sciences, Agricultural Research Organization ARO, the Volcani Center, Bet Dagan 50250, Israel

Strigolactones are newly identified plant hormones that regulate shoot and root development as well as plant interactions with parasitic plants and symbiotic microbes. An interesting aspect of strigolactones is their potential use in agriculture. A number of publications have discussed their implementation as inducer of suicidal seed germination of parasitic plant. However, strigolactones may be used in additional approaches for the development of new agricultural methodologies and technologies, compatible with emerging concepts of sustainable agriculture. For example, strigolactones may be used for improvement of root-system architecture, important for enhancing symbiotic interactions. Today new strigolactone analogs and mimics, which are under development or being synthesized, some of them specific for one activity, are likely to substantially promote the ability to use strigolactones to the benefit of agriculture. Potential utilization of these molecules in agriculture will be discussed.

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Mycorrhization for combating parasitic weeds: Approaches to control strigolactone germination stimulant exudation

MICHAEL H. WALTER, RON STAUDER, ALAIN TISSIER

Leibniz-Institute of Plant Biochemistry, Department of Cell & Metabolic Biology, D-06120 Halle (Saale), Germany

Seeds of the root-parasitic weeds *Striga* spp. and *Orobanche* spp. germinate only upon receiving chemical signals from their host root. Such signals are strigolactones exuded from roots as an ancient cue for promoting root colonization by symbiotic arbuscular mycorrhizal (AM) fungi, which is thought to have later been hijacked by the parasites to recognize host roots. It has been shown in several plant species that mycorrhization reduces infestation by these root parasites correlated with reduced levels of strigolactone root content and exudation. However, it is not clear, whether this effect is due to reduced strigolactone production or enhanced degradation or both. We have determined transcript levels of strigolactone biosynthetic enzymes and a strigolactone transporter and found them to be elevated even at later stages of the symbiosis arguing for a contribution of an AM-fungus-mediated strigolactone catabolism to its reduced levels in mycorrhizal roots. Such a catabolism might be analogous to the strigolactone perception process by the plant, which involves cleavage by a hydrolase. Understanding the mechanism of reduction of parasite infestations by AM fungi may allow to better exploit the biocontrol activity of mycorrhization. Another approach to lower exudation of germination stimulants is targeting strigolactone biosynthesis and particularly steps, which specifically contribute to strigolactone root exudation rather than to plant-internal phytohormone functions. It is argued that sustained exudation of strigolactones into the rhizosphere will require a regulated supply of carotenoid precursors to strigolactone apocarotenoid production. A specific isogene (PSY3) for a key step of carotenogenesis (phytoene synthase) has been identified, which is exclusively regulated in roots by phosphate starvation and mycorrhization. Knockdown of PSY3 gene expression in hairy roots of *Medicago truncatula* has resulted in reduced levels of mycorrhiza-induced root apocarotenoids of the C13 alpha-ionol and C14 mycorradicin type. Determination of strigolactone levels in these lines is currently underway.

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Name, Contact: Rozpądek Piotr, piotr.rozpadek@uj.edu.pl

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Strigolactone deficient *Arabidopsis thaliana* mutants max1 and max4 are hindered in their ability to form a symbiotic association with the endophytic fungus *Mucor plumbeus*

ROZPĄDEK P.[1,2], WAŻNY R.[4], WĘŻOWICZ K.[1], DOMKA A.[1], WICIARZ M.[3], TURNAU K.[1,4]

[1] *Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Kraków, Poland*
[2] *Institute of Plant Physiology, Polish Academy of Sciences, Niezapominajek 21, 30-239 Kraków, Poland*
[3] *Faculty of Biochemistry, Biophysics and Biotechnology*

In their natural environments plants interact with a multitude of microorganisms. The most common are fungi and bacteria, that symbiotically associate with plants in beneficial (mutualism), neutral (commensalism) or deleterious (parasitism) interactions. Symbiosis requires a number of adjustments leading to the establishment of a new metabolic homeostasis between the plant host and its partner. Various egzo- or endogenous cues may disturb this equilibrium causing endophytes (mutualists and commensals) to become parasites and vice-versa. In fact, disease may be regarded as an unbalanced state of symbiosis. The molecular and biochemical factors determining this balance are not known, however recently, significant progress has been made in elucidating the mechanisms of plant-endophyte versus plant parasite interactions and key factors playing a role in the “switch” from endophytic to parasitic lifestyles have been recognized. Strigolactones (SL) have been shown to facilitate the beneficial interaction between plants and mycorrhizal fungi. In this study we tested the role of SL in the interaction between *A. thaliana* and the fungal endophyte *Mucor plumbeus*. We hypothesized that SL may participate in the cross-talk between the plant and its endophytic partner playing a role in determining this interaction mutualistic. *Mucor plumbeus* was isolated from *Arabidopsis arenosa* from a post-industrial waste in close proximity to Kraków, Poland. *Arabidopsis thaliana* WT and SL biosynthetic mutants (max1 and max4) were inoculated with *M. plumbeus* and grown in in-vitro cultures. Inoculation resulted in growth impairment of SL deficient mutants. This corresponded with a significant up-regulation of biotic stress defense related genes (PR1, PR2, PR3, PR5, PDF1.2, ERF1). The development along with the respiration rate of *Mucor plumbeus* grown in PDA medium supplemented with the synthetic SL analog GR24 was also affected. According to our results SL influences the development of *M. racemosus* and its interaction with its host *A. thaliana*.

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Specificity in plant - arbuscular mycorrhizal fungal (AMF) interactions; reality or a fata morgana?

THEO RUISSEN

Norwegian Institute for Agricultural and Environmental Research, Gunnars veg 6, 6630 Tingvoll, Norway.

Host specificity in plant-fungus interactions is commonly present and not at least well known from the field of fungal plant pathogens. The intimate interactions of arbuscular mycorrhizal fungi, already present from the dawn of plant life on earth, show a more complex picture with regard to host specificity. This despite the fact that the issue is under debate for almost half a century.In this presentation, an overview is given of the present state-of-the-art in this area. Evidence supporting specificity, but also uncertainty in our insight will be presented. Crucial elements as for example the possible underlying mechanisms- among them strigolactone signalling- and research methodological approaches used in deciphering the phenomenon will be highlighted and discussed.

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Functional genomics tools to investigate fungal responses to strigolactones

BELMONDO S.[1], MARSCHALL R.[2], SCHUMACHER J.[2], TUDZYNSKI P.[2], ARTUSO E.[3], PRANDI C.[3], LANFRANCO L.[1]

[1] *Department of Life Sciences and Systems Biology, University of Torino, Torino (Italy)*
[2] *Institut für Biologie und Biotechnologie der Pflanzen, Westf. Wilhelms-Universität Münster, Münster (Germany)*
[3] *Department of Chemistry, University of Torino, Torino (Italy)*

Strigolactones (SLs) as components of root exudates induce hyphal branching of arbuscular mycorrhizal fungi (AMF), an event which is thought to favor root colonization and the establishment of the mutualistic symbiosis. Little is known on how AMF detect and respond to SLs. AMF are a poor model system to investigate SLs action due to the obligate biotrophism and the lack of genetic transformation protocols. Moreover, SLs are widely distributed in the plant kingdom and this supports the hypothesis that SLs also have other important roles possibly affecting both beneficial and pathogenic microorganisms. It has been shown that a number of phytopathogenic fungi, including the well-studied *Botrytis cinerea*, are sensitive to GR24, a synthetic analog SLs and a reference molecule in SLs research. With the aim to identify the molecular determinants involved in SLs response in AMF and assuming conserved mechanisms of SLs response in the fungal kingdom, we exploited *B. cinerea*, for which genomics data and mutant collections are available. Exposure of *B. cinerea* to GR24 led to a reduction in radial growth and an altered hyphal morphology with a slight increased branching. We therefore set up the screening of a collection of *B. cinerea* knock-out mutants, defective in genes mainly involved in signalling to isolate genotypes with altered responses to SLs. Among the 40 mutant strains analysed, three turned out to be less responsive to GR24: one mutant is defective of a G gamma subunit of heterotrimeric GTPases, which are known as main components of the signal transduction pathway; the second strain is defective of a thioredoxin reductase (trr) which has a key role in the control of cell redox omeostasis by forming reduced disulfide bonds; the third is defective of a GATA transcription factor. One feature shared by the three mutants is the overproduction of ROS (reactive oxygen species). Analyses on *B. cinerea* wt strain expressing a redox sensitive-GFP showed that GR24 induces changes in the redox status and, in particular, an oxidizing effect at the level of mitochondria. These data suggest that the response to GR24 requires changes of the redox status. We envisage that exploitation of this fungal system can be instrumental for the identification of cellular and molecular mechanisms of fungal responses to SLs.

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Inoculation of *Lactuca serriola* with endophytic and mycorrhizal fungi improves phytoremediation

RAFAŁ WAŻNY[1], PIOTR ROZPĄDEK[2,3], JĘDRZEJCZYK ROMAN[1], WERONIKA JANAS[3], KATARZYNA TURNAU[1,3]

[1] *Malopolska Centre of Biotechnology, Jagiellonian University, Kraków, Poland*
[2] *Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland*
[3] *Institute of Environmental Sciences, Jagiellonian University, Kraków, Poland*

Phytoremediation is an eco-friendly technology for treatment of the toxic metal polluted soils. To improve the efficiency of phytoremediation, both mycorrhizal fungi and endophytes can be utilized. These microorganisms improve plant fitness and growth by alleviating the effects of metal toxicity. In our experiment we tested wild lettuce (*Lactuca serriola*), considered as a pioneer of open habitats, tolerant to low moisture and poor nutrient conditions. Seedlings inoculated with the AM fungus *Rhizophagus intraradices* and endophytic fungi were grown in polluted substratum. Control treatments were grown in garden soil and sand mixture. Biometric parameters, chlorophyll and flavonoid content and chlorophyll a fluorescence in leaves, fungal colonization of roots and toxic metal accumulation were analyzed. Inoculation with *Rhizophagus intraradices* enhanced plant growth in both toxic metal enriched and control substrata. The roots were abundantly colonized by mycorrhizal fungus. Colonization by endophytes had a positive effect on biomass production.

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Participants and contacts

Name	Contact
Abdelaziz Heddi	**Abdelaziz.Heddi@insa-lyon.fr**
Agathe Lecointe	**agathe.lecointe@epfl.ch**
Aileen Berasategui	**aberasategui@ice.mpg.de**
Ailsa McLean	**ailsa.mclean@zoo.ox.ac.uk**
Alejandro Manzano Marin	**alejandro.manzano.marin@gmail.com**
Alessandra Pepe	**alessandra.pepe@for.unipi.it**
Allison Kerwin	**allison.kerwin@uconn.edu**
Amelia Lindsey	**alind005@ucr.edu**
Amornrat Chaiyasen	**amornrat057@gmail.com**
Ana Corrêa	**ambcorrea@gmail.com**
Ana Regueiras	**aregueiras@ciimar.up.pt**
Ana Soares	**ana_afss@hotmail.com**
Anders Meibom	**anders.meibom@epfl.ch**
Andrea Genre	**andrea.genre@unito.it**
Andreas Beck	**beck@bsm.mwn.de**
Andrès Gutierrez Rodriguez	**agutierrez@sb-roscoff.fr**
Andres Sadowsky	**Andres.Sadowsky@hhu.de**
Angela Figás Segura	**Angela.Figas@uv.es**
Anna Voytsekhovich	**trebouxia@gmail.com**
Anna Zaidman-Remy	**anna.zaidman@insa-lyon.fr**
Annette Brandt	**annette.brandt@hhu.de**
Anthony E. Glenn	**anthony.glenn@ars.usda.gov**
António Manso	**manso.ipt@gmail.com**
Arantxa Molins	**arantxa.molins@uv.es**
Arnaud Tanguy	**atanguy@sb-roscoff.fr**
Aurélie Tasiemski	**aurelie.tasiemski@univ-lille1.fr**
B. Mariana Reyes-Prieto	**bertmare@uv.es**
Briony Barr	**scalefreenetwork@gmail.com**
Camille Detree	**cdetree@sb-roscoff.fr**

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Carolyn A. Young	**cayoung@noble.org**
Catarina Gouveia	**catarina.a.gouveia@gmail.com**
Catherine Masson	**Catherine.Masson@toulouse.inra.fr**
Cecile Sabourault	**Cecile.Sabourault@unice.fr**
Cessa Rauch	**cessa.rauch@hhu.de**
Charles W. Bacon	**charles.bacon@ars.usda.gov**
Christian Printzen	**cprintzen@senckenberg.de**
Christoph Vorburger	**christoph.vorburger@eawag.ch**
Christophe Roux	**roux@lrsv.ups-tlse.fr**
Claudia Vannini	**claudia.vannini@unipi.it**
Clinton Oakley	**clint.oakley@vuw.ac.nz**
Cristiana Sbrana	**sbrana@ibba.cnr.it**
Cristina Cruz	**ccruz@fc.ul.pt**
Cristina Prandi	**cristina.prandi@unito.it**
Dani Vincent	**vincent.dani@unice.fr**
Daniel Martin	**dani@ceab.csic.es**
David Chareles Queller	**queller@wustl.edu**
David Giron	**david.giron@univ-tours.fr**
David Richardson	**david.richardson@smu.ca**
Davide Vecchi	**davide.s.vecchi@gmail.com**
Debbie Brock	**dbrock@wustl.edu**
Diogo Neves Proença	**diogo.proenca@gmail.com**
Dorion Sagan	
Elad Chiel	**eladchiel@gmail.com**
Eleanor Heyworth	**eh542@york.ac.uk**
Elfie Stocker-Wörgötter	**elfiestocker@gmx.at**
Élio Sucena	**eliosucena@gmail.com**
Elizabeth Ann Czerwinski	**eczerwin@umail.iu.edu**
Enric Frago	**enric.frago@gmail.com**
Eric Bapteste	**eric.bapteste@upmc.fr**
Erik F. Y. Hom	**erik@olemiss.edu**

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Participants and contacts

Ernesto Alejandro Zavala Gonzalez	**ibq.zavala@gmail.com**
Eva Barreno	**eva.barreno@uv.es**
Ewa Chrostek	**echrostek@igc.gulbenkian.pt**
Fabrice Not	**not@sb-roscoff.fr**
Febri Doni	**doni@siswa.ukm.edu.my**
Filip Husnik	**filip.husnik@gmail.com**
Flore Zélé	**zeleflore@gmail.com**
Florence Gutzwiller	**florence.gutzwiller@gmail.com**
Florent Masson	**florent.masson@insa-lyon.fr**
Florian Ulm	**ulm.florian@gmail.com**
Francisco Carrapiço	**fcarrapico@fc.ul.pt**
Francisco Cerqueira	**diogoalmeida7@hotmail.com**
Francisco Dionísio	**fadionisio@fc.ul.p**
François H. Lallier	**lallier@sb-roscoff.fr**
Frederique Guinel	**fguinel@wlu.ca**
Fredrick Lee	**fjlee@indiana.edu**
Girish Beedessee	**girish.beedessee@oist.jp**
Graziella Berta	**graziella.berta@uniupo.it**
Gregory Crocetti	**gregory@scalefreenetwork.com.au**
Gulnar A. Akhtemova	**ahgulya@yandex.ru**
Hinanit Koltai	**hkoltai@agri.gov.il**
Ian Probert	**probert@sb-roscoff.fr**
Inês de Sousa Rocha	**ines.sousa.rocha@gmail.com**
Ioana Onut Brännström	**ioana.brannstrom@ebc.uu.se**
Irene Newton	**irnewton@indiana.edu**
Irshad-Ul Haq	**i.u.haq@rug.nl**
Isabel Maria de Oliveira Abrantes	**isabel.abrantes@zoo.uc.pt**
Jacqueline Baar	**jacqueline@biomygreen.com**
Jacques Batut	**jacques.batut@toulouse.inra.fr**
James F. White	**white@rci.rutgers.edu**
Jan de Vries	**jan.vries@hhu.de**

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Index

Jan Dirk Van Elsas	**j.d.van.elsas@rug.nl**
Jan Sapp	**jsapp@yorku.ca**
Jana Steinová	**jana.steinova@gmail.com**
Janusz Blaszkowski	**janusz.blaszkowski@zut.edu.pl**
Jaturong Kumla	**Jaturong_yai@hotmail.com**
Jessica Morera Huertas	**jesmorera@gmail.com**
Jillian Petersen	**jmpeters@mpi-bremen.de**
Joachim Meessen	**joachimmeessen@gmx.de**
Joan Elizabeth Strassmann	**strassmann@wustl.edu**
Joana Falcao Salles	**j.falcao.salles@rug.nl**
João Coelho	**joao.coelho595@gmail.com**
João Tavares	**tavaresjeb@gmail.com**
Johana Revel	**jrevel@unice.fr**
John F. Brooks	**z5x1y1@u.northwestern.edu**
John McMullen II	**jgm2@email.arizona.edu**
Jon Seal	**jon.seal@utexas.edu**
Juliana Melo da Conceição	**julianas2m@hotmail.com**
Kamil M. Szafranski	**kamil.szafranski@u-psud.fr**
Kamran Azmaliyev	**azmaliyev.kamran@hotmail.com**
Karin Pritsch	**pritsch@helmholtz-muenchen.de**
Karina Xavier	**kxavier@igc.gulbenkian.pt**
Katarzyna Turnau	**katarzyna.turnau@uj.edu.pl**
Katherine Elizabeth Muller	**mulle374@umn.edu**
Kathy Sheehan	**sheehaka@gmail.com**
Katrin Kellner	**antkatrina@gmail.com**
Kouki Uchinomiya	**uchinomiya@bio-math10.biology.kyushu-u.ac.jp**
Kumkum Azad	**kumkum.azad@usask.ca**
Laura Runyen-Janecky	**Lrunyenj@richmond.edu**
Laura S. Weyrich	**laura.weyrich@adelaide.edu.au**
Le Blao Simon	**simon.le.bloa@ifremer.fr**

Back to Index

Lisl Esherick	**lisl.esherick@stanford.edu**
Liu Yin-Quan	**yqliu@zju.edu.cn**
Louis S. Tisa	**louis.tisa@unh.edu**
Louis Yohan	**yo_han20@hotmail.com**
Lucia Muggia	**lmuggia@units.it**
Lucie Van?urová	**lucie.vancurova@natur.cuni.cz**
Luis Carvalho	**lmcarvalho@fc.ul.pt**
Luís Correia	**Luis.Correia@ciencias.ulisboa.pt**
Luis Manuel Bolaños	**lbolanos@lcg.unam.mx**
Luis Teixeira	**lteixeira@igc.gulbenkian.pt**
Luis Vicente Lopez-Llorca	**lv.lopez@ua.es**
Mahmoudi Néjí	**neji.2012@jahoo.com**
Manju M. Gupta	**mbansalsac@outlook.com**
Marc-André Selosse	**ma.selosse@wanado.fr**
Marcia González-Teuber	**mfgonzalez@userena.cl**
Marco A. Villanueva	**marco@cmarl.unam.mx**
Marco Cosme	**cosme@zedat.fu-berlin.de**
Maria Clara Vieira Santos	**mcvs@sapo.pt**
Maria Cristina M. Motta	**motta@biof.ufrj.br**
María de Lourdes Adriano Anaya	**msalvad@hotmail.com**
María J. Pozo	**mjpozo@eez.csic.es**
Maria Manuela Lemos Carolino	**mmcarolino@fc.ul.pt**
Marie Anne Cambon Bonavita	**macambon@ifremer.fr**
Mariya V. Zhukova	**mariya.zhukova@bio.ku.dk**
Mark Blaxter	**mark.blaxter@ed.ac.uk**
Marta Salgueiro Alves	**martasalgueiroalves@gmail.com**
Martha Kruchten	**martha_kruchten@hotmail.com**
Martin Kaltenpoth	**mkaltenpoth@ice.mpg.de**
Mary Beth Saffo	**mbsaffo@post.harvard.edu**
Mary Jean	**jmary@sb-roscoff.fr**
Maryam Chaib De Mares	**m.chaib.de.mares@rug.nl**

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Participants and contacts

Marzena Sujkowska-Rybkowska	marzena_sujkowska@sggw.pl
Melanie Smee	melanie.smee@york.ac.uk
Mélisandre Téfit	melisandre.tefit@ens-lyon.fr
Melissa Whitaker	melissawhitaker@fas.harvard.edu
Mercedes Berlanga	mberlanga@ub.edu
Michael Walter	mhwalter@ipb-halle.de
Miguel Beltran-Garcia	mbeltran71@hotmail.com
Myriam Catalá	myriam.catala@urjc.es
Nathalie Gontier	nlgontier@fc.ul.pt
Naveen Kumar Arora	nkarora_net@rediffmail.com
Nicole Lopanik	nlopanik@gsu.edu
Nuria Escudero	nuria.escuder@gmail.com
Oksana Shtark	oshtark@yandex.ru
Olivier Detournay	odetournay@gmail.com
Ondrej Peksa	opeksa@zcm.cz
Paola Furla	furla@unice.fr
Patricia Correia	pat_correia@yahoo.com
Patricia Moya	patricia.moya@uv.es
Patricia Stock	spstock@email.arizona.edu
Patrícia Ventura	tixa.ventura@gmail.com
Paula Vasconcelos Morais	pvmorais@ci.uc.pt
Pedro Miguel Silva	
Piotr Rozpadek	piotr.rozpadek@uj.edu.pl
Polina Dgebuadze	p.dgebuadze@gmail.com
Pu Yang	p.yang@rug.nl
Rafal Wazny	rafal.wazny@uj.edu.pl
Ranjeet Bhagooli	r.bhagooli@uom.ac.mu
Regina Redman	rsredman@adsymtech.com
Ricardo Guerrero	rguerrero@iec.cat
Rui S. Oliveira	rsoliveira@uc.pt
Rupinder Kaur	rimzz.mht@gmail.com

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Rusty Rodriguez	**rjrodriguez@adsymtech.com**
Saisamorn Lumyong	**scboi009@gmail.com**
Samantha Fernandez Brime	**Samantha.Fernandez@nrm.se**
Sara Magalhães	**snmagalhaes@fc.ul.pt**
Satoshi Nakagawa	**nsatoshi@kais.kyoto-u.ac.jp**
Sebastian Baumgarten	**sebastian.baumgarten@kaust.edu.sa**
Sebastien Duperron	**sebastien.duperron@snv.jussieu.fr**
Sergio Enrico Favero Longo	**sergio.favero@unito.it**
Sevda Haghi Kia	**haghikia@stud.uni-frankfurt.de**
Sheila Kitchen	**kitchens@science.oregonstate.edu**
Sieglinde Ott	**otts@hhu.de**
Silvana Munzi	**ssmunzi@fc.ul.pt**
Silvia Bulgheresi	**silvia.bulgheresi@univie.ac.at**
Silvia Libro	**libro@neb.com**
Simon Davy	**simon.davy@vuw.ac.nz**
Simone Belmondo	**simone.belmondo@unito.it**
Sofia Laura Ferreira Leite	**slleite@ua.pt**
Sónia Duarte	**smpduarte@gmail.com**
Sophie Mai Colston	**sophie.colston@uconn.edu**
Spencer Nyholm	**spencer.nyholm@uconn.edu**
Stanley Freeman	**freeman@volcani.agri.gov.il**
Stephanie Barnay-Verdier	**stephanie.barnay-verdier@upmc.fr**
Stephanie Markert	**stephanie.markert@uni-greifswald.de**
Stergios-Orestis Kolokotronis	**skolokotronis@fordham.edu**
Susana Castro	**s.castro.sow@gmail.com**
Sushma Mattan-Moorgawa	**s.moorgawa@uom.ac.mu**
Sven Gould	**gould@hhu.de**
Szymon Zubek	**szymon.zubek@uj.edu.pl**
Temir Britayev	**britayev@yandex.ru**
Teresa Dias	**mtdias@fc.ul.pt**
Teresa Lino-Neto	**tlneto@bio.uminho.pt**

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Tereza ?idká	**tereza.ridka@seznam.cz**
Tetiana Lutsak	**luts.ok@gmail.com**
Theo Ruissen	**theo.ruissen@bioforsk.no**
Thomas Fester	**tfester@scivit.de**
Thomas Krueger	**thomas.krueger@epfl.ch**
Thomas Ogao Onchuru	**tonchuru@ice.mpg.de**
Thorsten Grams	**grams@tum.de**
Tjorven Hinzke	**tjorven.hinzke@stud.uni-greifswald.de**
Tobias Engl	**tengl@ice.mpg.de**
Tsubasa Ohbayashi	**tsubasa-oobayashi@aist.go.jp**
Ulla Kaasalainen	**ulla.kaasalainen@geo.uni-goettingen.de**
Ulrike Ruprecht	**ulrike.ruprecht@sbg.ac.at**
Vanessa Aparecida Chagas Moutinho	**chagasmoutinho@gmail.com**
Veena Pande	**veena_kumaun@yahoo.co.in**
Virginia Weis	**weisv@science.oregonstate.edu**
Vitor Faria	**vfaria@igc.gulbenkian.pt**
Viviane Radl	**viviane.radl@helmholtz-muenchen.de**
Wieslaw J. Kozek	**wieslaw.kozek@upr.edu**
William B. Sanders	**wsanders@fgcu.edu**
Wolfgang Miller	**wolfgang.miller@meduniwien.ac.at**
Yin Wang	**yin.wang9598@gmail.com**
Yoav Soen	**yoavs@weizmann.ac.il**
Yolande Dalpé	**yolande.dalpe@agr.gc.ca**
Yoram Kapulnik	**kapulnik@agri.gov.il**
Yoshitomo Kikuchi	**y-kikuchi@aist.go.jp**
Yulia Gnainsky	**yulia.gnainsky@weizmann.ac.il**
Zakia Boubakir	**zakia.boubakir@usask.ca**
Zerrin Uzum	**zerrin.uzum@leibniz-jena.de**

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Conference Dinner: “Casa do Alentejo”

Tuesday 14 July at 20:00

R. das Portas de Santo Antão 58, 1150 Lisboa

Phone: +351 213405140

Map:

How to find it:

The easiest way to reach the restaurant is by taxi, if you are 4 people in each taxi, the cost per person will be slightly higher than the cost of the metro ticket. By metro, take the blue line (linha azul) in direction to “Santa Apolonia” and get out at the “Restauradores” station. Then, walk two minutes following the map.

Menu:

Appetizer: Bread, olives, sausages from Alentejo.
Soup: Carrot soup with coriander.
Meat: Pork meat “á Alentejana” (with clams); dessert: eggs pudding.
Fish: Rice with monkfish and shrimps; dessert: eggs pudding.
Vegetarian: Vegetables with sweet and sour sauce; soy hamburger; dessert: fruit salad.
To drink: Water, wine from Alentejo (white and red), beer, soft drinks, and coffee.