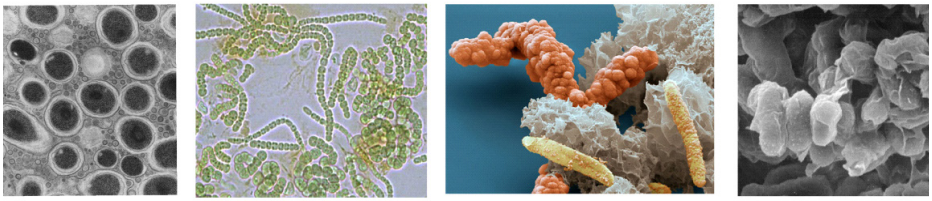

1. International RTG 1708 Workshop on "Bacterial survival strategies"

Bad Urach, 30. Sept. – 2. Oct. 2013

EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Greeting

Dear Participants,

It´s our pleasure to welcome you to the First International RTG 1708 Workshop on "Bacterial Survival Strategies" in Bad Urach, Germany.

Bacteria acquired during evolution elaborated strategies to withstand and overcome unfavourable conditions. These processes are fundamental for bacteria to protect their niches and colonize new habitats. Therefore, this issue is of highest relevance in bacterial ecology, physiology and medicine, e.g. for understanding the dispersal of bacterial pathogens and for the development of new antimicrobial drugs. The research training group (RTG) provides a new interdisciplinary research and graduate-training/qualification platform for fundamental microbiology in Tübingen.

The First International RTG 1708 Symposium on "Bacterial Survival Strategies" covers a broad range of topics in microbiology research. Oral presentations, selected short talks, posters and a small walking tour will offer plenty of opportunities for senior and junior scientists to learn about advances and to discuss them.

We thank our sponsors for supporting this basic research workshop and are looking forward to an interesting and stimulating workshop.

On behalf of The Organizing Committee

Alexander Klotz, Lehrstuhl Organismische Interaktionen
Dorothee Weisbrod, Institut für organische Chemie
Annika Schmidt, Institut für medizinische Mikrobiologie und Hygiene
Marlene Röttgen, RTG Geschäftstelle
Karl Forchhammer, Lehrstuhl Organismische Interaktionen

Program

Monday 30 September 2013

14:00 – 14:30	**Arrival and Room occupancy**
14:30 – 15:00	**Opening (Prof. Forchhammer, Organizing Committee)**
15:00 – 18:00	**Symposium 1** **Chair: Prof. Dr. Hantke**
15:00 – 15:30	Prof. Dr. Andreas Brune, Marburg
15:30 – 16:00	Prof. Dr. Christian Kost, Jena
16:00 – 16:15	Samay Pande, Jena
16:15 – 16:30	**Coffee break**
16:30 – 17:00	Prof. Dr. Ralf J. Sommer, Tübingen
17:00 – 17:15	Igor Iatsenko, Tübingen
17:15 – 17:45	Prof. Dr. Helge B. Bode, Frankfurt
17:45 – 18.00	Darko Kresovic, Frankfurt
18:00 – 19:00	**Dinner**
19:00 – 20:30	**Poster Session 1**
19:00 – 19:15:	Flash Poster Session: 1 Isabell Flade, Tübingen 2 Anna Lange, Tübingen 3 Alexander Zipperer, Tübingen 4 Shilpa George, Tübingen 5 Ali Coskun, Tübingen 6 Simon van Vliet, Zürich
19:15 – 20:30	Poster viewing
20:30-	**Get together**

Tuesday 1 October 2013

08:00 – 09:00	**Breakfast**
09:00 – 12:00	**Symposium 2** **Chair: Prof. Dr. Christiane Wolz**
09:00 – 09:30	Prof. Dr. Stephen Lory, Harvard, USA
09:30 – 10:00	Prof. Dr. Dieter Haas, Lausanne, Switzerland
10:00 – 10:15	Annika Schmidt, Tübingen
10:15 – 10:45	**Coffee break**
10:45 – 11:15	Prof. Dr. Julia Frick, Tübingen
11:15 – 11:45	Dr. Bernhard Krismer, Tübingen
11:45 – 12:00	Sonja Mayer, Tübingen
12:00 – 13:00	**Lunch**
13:00 – 16:00	**Group Photo and Small Walking Tour**
16:00 –18:00	**Poster Session 2**
16:00 – 16.20	Flash Poster Session 7 Bastian Blauenburg, Marburg 8 Andreas Mielcarek, Marburg 9 Sabrina Rohrer, Tübingen 10 Waldemar Hauf, Tübingen 11 Alexander Klotz, Tübingen 12 Klaus Brilisauer, Tübingen 13 Aris-Edda Stachler, Ulm
16:20 – 18:00	Poster viewing
18:00 – 19:00	**Dinner**
19:00 – 20:30	**Symposium 3** **Chair: PD Dr. Iris Maldener**
19:00 – 19:30	Prof. Dr. Elke Dittmann, Potsdam
19:30 – 20:00	Prof. Dr. Frank Schreiber, Zürich
20:00 – 20:15	Dorothee Weisbrod, Tübingen
20:15 – 20:30	Sven Meisner, Potsdam
20:30 -	**Get together**

Wednesday 2 October 2013

08:00 – 09:00	**Breakfast**
09:00 – 12:00	**Symposium 4** **Chair: Prof. Dr. Friedrich Götz**
09:00 – 09:30	Prof. Dr. Sonja-Verena Albers, Marburg
09:30 – 09:45	Benjamin Meyer, Marburg
09:45 – 10:15	Prof. Dr. Anita Marchfelder, Ulm
10:15 – 10:30	Carsten Dietrich, Marburg
10:30 – 11:00	**Coffee break**
11:00 – 11:15	Kristina Paul, Marburg
11:15 – 11:45	Prof. Dr. Andreas Kappler, Tübingen
11:45 – 12:00	Tina Gauger, Tübingen
12:00 – 13:00	**Lunch**
13:00 – 15:00	**Symposium 5** **Chair: Prof. Dr. Stephanie Grond**
13:00 – 13:15	Enrico Muhr, Marburg
13:15 – 13:30	Lina Clermont, Marburg
13:30 – 13:45	Rebeca Perez, Tübingen
13:45 – 14:15	Prof. Dr. Karl Forchhammer, Tübingen
14:15 – 14:30	**Coffee break**
14:30 – 15:00	Round Table Discussion
15:00 – 15:15	**Closing Remarks** **(Prof. Forchhammer,** **Organizing Committee)**

1. International RTG 1708 Workshop on "Bacterial survival strategies"

Abstracts

Evolutionary origin of the symbiotic microbiota in termite guts

Andreas Brune

Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

The recent finding of termite-specific bacterial lineages in the gut of cockroaches prompts the hypothesis that the symbiotic gut microbiota of termites is derived from a common ancestor. However, the microbial diversity in many termite families has not been fully explored, and even less is known about that in cockroaches. We characterized the bacterial communities in the hindguts of 34 host species by pyrotag sequencing of the 16S rRNA gene (V3–V4 region) using a modified primer set. A comprehensive analysis based both on phylogeny and hierarchical classification revealed that the gut microbiota of termites is less diverse and more specialized than that of cockroaches. The gut communities of different host lineages formed distinct clusters. Although community structure differed strongly already at the phylum level, we identified numerous genus-level taxa that were present in almost every host. Remarkable changes in their relative abundance correlate with major events in the evolutionary history of termites, such as acquisition and loss of cellulolytic flagellates or dietary diversification. The emerging patterns document a long history of co-evolution of termites and their gut microbiota, which provided a reservoir of bacterial diversity that was exploited whenever new functions were required.

Less is more – Bacterial gene loss results in division of labor and the formation of intercellular networks

Christian Kost[1,2]

[1]Research Group Experimental Ecology and Evolution, Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena;
[2]Institute of Microbiology - Microbial Phytopathology, Friedrich Schiller University Jena, Jena

Cooperative cross-feeding interactions, in which bacterial cells exchange metabolites to the benefit of the interacting partners, are very common in the microbial world. Despite their fundamental ecological importance, does the existence of these interactions pose an evolutionary conundrum: Why does one organism invest costly resources into another one and not use them for itself. We address this question by combining approaches from synthetic ecology and experimental evolution to identify the ecological factors and evolutionary conditions that promote the evolution and maintenance of metabolic cross-feeding interactions.

By simply deleting two metabolic genes, we generated a range of genotypes that reciprocally exchanged essential amino acids when cocultured. Surprisingly, in a vast majority of cases, cocultures of two of these cross-feeding strains showed an increased Darwinian fitness relative to unmutated wild type (WT) cells – even in direct competition. This unexpected growth advantage was due to a division of metabolic labour among cooperating cells: the fitness cost of overproducing certain amino acids was less than the

energetic gain of not having to produce others when they were provided by their partner. Moreover, these metabolic cross-feeding interactions were resistant to the invasion of noncooperating genotypes and readily evolved in selection experiments.

Further extending this analysis revealed i) metabolic auxotrophies are very common in nature, ii) bacteria commonly release significant amounts of amino acids during growth, and iii) the loss of metabolic genes can be adaptive when the focal metabolite is sufficiently present in the environment.

Based on these results I develop a model in which cross-feeding of essential metabolites is a very prevalent survival strategy used by bacteria. Ultimately, our findings provide an adaptive explanation for the ease, with which bacteria enter into metabolic mutualisms with other micro- or macroorganisms and suggest bacteria most likely function as a network of interacting cells, rather than as physiologically autonomous units.

It takes two to tango: Phenotypic dissimilarity promotes cooperation in bacterial cross-feeding interactions

Samay Pande[1], Katrin Bohl[2], Sebastian Germerodt[2], Stefan Schuster[2], Christian Kost[1]

[1]Experimental Ecology and Evolution Research Group, Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany,
[2]Department of bioinformatics, Friedrich University Jena, Jena, Germany

Explaining the evolution and maintenance of cooperative interactions is a major problem in evolutionary biology. Here, the main challenge lies in understanding why one individual produces resources that benefit another individual at a cost to itself. A solution to this paradox has been offered by Hamilton, who argued indirect fitness benefits amongst close relatives could explain the existence of such interactions. This principle, however, does not apply to interspecific interactions, where the genetic relatedness between partners is low. Nevertheless, interspecific cooperative interactions are pervasive in nature. Especially microorganisms tend to live in multispecies environments, where they frequently enter into cooperative interactions with individuals from different species.

To understand the reasons for the existence and stability of such interactions, we synthetically constructed obligate amino acid cross-feeding interactions within (*Escherichia coli*) and between (*Escherichia coli – Acinetobacter baylyi*) species. Surprisingly, monitoring the population dynamics of these interactions in the presence and absence of non-cooperating individuals showed that between-species interactions were more stable than within-species interactions. Non-cooperators and cooperators showed a stable co-existence and - in contrast to evolutionary theory - did not collapse in the presence of non-cooperating types. Moreover, our results showed that the cost of cooperation was negligible, thus explaining the ease with which microbes enter into cooperative interactions. Finally, the interacting partners differed in their uptake kinetics of amino acid, which could explain the long-term stability of these interactions.

Taken together, our results demonstrate that a phenotypic dissimilarity between two cooperating individuals can stabilize cooperative interactions, even when the interacting partners show a low genetic relatedness

Reciprocal genetics of *Caenorhabditis elegans – Bacillus* interactions reveals insight into virulence and resistance mechanisms

Igor Iatsenko, Ralf J. Sommer

Max Planck Institute for Developmental Biology, Department for Evolutionary Biology

Genetically tractable model organisms *C. elegans* and *Pristionchus pacificus* have been successfully used in the host-pathogen interaction studies and contributed to the understanding of the innate immunity function and uncovered conserved virulence factors of clinically relevant pathogens. At the same time interactions of these nematodes with their natural pathogens are poorly investigated. Given that *Bacillus* bacteria often coexist with the nematodes and some of them are known pathogens of invertebrates, previously we isolated 768 *Bacillus* strains and tested them for the virulence to nematodes. Although only 3% of tested *Bacillus* were pathogenic, one strain called *B. thuringiensis* DB27 exhibited extreme virulence to *C. elegans* but not to *P. pacificus*. Currently we are trying to identify virulence factors of this pathogen and also nematode defense mechanisms. First, we discovered that virulence factors of DB27 are plasmid-encoded and most likely are Cry toxins, since plasmid-cured mutants are not pathogenic and do not produce Cry toxins. Whole genome sequencing of DB27 indeed showed presence of novel nematocidal Cry toxin. We expressed this novel toxin in E. coli and confirmed its activity against *C. elegans*. Importantly, this toxin is also active against other free-living and animal parasitic nematodes, suggesting potential application against parasitic nematodes. Our parallel work on the host side led to discovery of *C. elegans* novel pathway involved in the defense against DB27. Specifically, we identified novel function for Dicer in *C. elegans* antibacterial innate immunity and showed that this function is largely associated with microRNA processing. Taken together, our reciprocal studies uncovered a previously unknown role for DCR-1/Dicer in *C. elegans* antibacterial immunity as well as identified novel nematocidal toxin.

Small talk in *Photorhabdus* and *Xenorhabdus*: Signals, toxins, antibiotics!

Helge B. Bode

Merck Stiftungsprofessur für Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, Germany

Entomopathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus* live in symbiosis with nematodes of the genera *Heterorhabditis* and *Steinernema*, respectively, and have been shown to be prolific producers of natural products(1).A detailed analysis of 300 different strains of these bacteria grown without nematodes led to the identification of several natural products that are novel and often conserved in specific phylogenetic clades of these bacteria thus indicating also specific conserved functions of these compounds. Current efforts in our group deal with the activation and optimization of secondary metabolite production via the manipulation of specific or global regulators, the exchange of natural promoters against strong or inducible ones, and the production of compounds, which are specifically produced exclusively in insects. Among the novel compound classes identified during our work are several new nonribosomally made peptides or peptide derivatives, widespread but overlooked dialkylresorcinols and cyclohexanediones, pyrones acting as a novel bacterial signaling system, and structurally unique polyamine natural products. The major goal of our work is to elucidate the natural function of these compounds as well as the regulatory mechanisms that enable their production.

1. Bode, H. B., *Curr. Opin. Chem. Biol.* **2009**, *13,* 224.

Unusual ketosynthases from Photorhabdus and Xenorhabdus

Darko Kresovic

Merck Stiftungsprofessur für Molekulare Biotechnologie, Prof. Dr. Helge B. Bode, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, Germany

Generally ketosynthases are known to be essential for fatty acid and polyketide biosynthesis. They are either part of large multifunctional enzymes like the type I polyketide synthases (PKS) and type I fatty acid synthases, or they exist as stand-alone enzymes as in the type II fatty acid synthases, type II PKS, or chalcone synthases (type III PKS). During the search for new bioactive natural products from entomopathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus*, our group could elucidate the biosynthesis of several compounds, in which an unusual ketosynthase plays a crucial role. So is DarB forming the widespread but overlooked dialkylresorcinols and cyclohexanediones(1), XclC is involved in the biosynthesis of Xenocyloin and PpyS is catalysing the formation of photopyrones which act as a novel bacterial signaling system.(2)These ketosynthases differ in their reaction from known ones as they catalyze the cyclization of two fatty acid derivatives or act as an acyltransferase. By generating a database of ketosynthases including our unusual ketosynthases as well, we were able to conduct a detailed phylogenetic analysis. Thereby we could show that each of this newly identified ketosynthases forms a novel clade with ketosynthases from other organisms presumably also performing the unusual catalytic function. Thus, also the natural products derived from the respective biosynthesis all show interesting biological activities might therefore also be more widespread than originally thought.

1. S. W. Fuchs, K. A. Bozhüyük, D. Kresovic, F. Grundmann, V. Dill, A. O. Brachmann, N. R. Waterfield, H. B. Bode. Formation of 1,3-cyclohexanediones and resorcinols catalyzed by a widely occuring ketosynthase. *Angewandte Chemie (International ed. in English)* **2013**, *52*, 4108-12.
2. A. O. Brachmann, S. Brameyer, D. Kresovic, I. Hitkova, Y. Kopp, C. Manske, K. Schubert, H. B. Bode, R. Heermann. Pyrones as bacterial signaling molecules. *Nature chemical biology* **2013**, DOI 10.1038/nchembio.1295.

The dichotomic role of LPS: induction or prevention of intestinal inflammation

Kerstin Gronbach[1, 12#], Isabell Flade[1, 12#], Otto Holst[2], Buko Lindner[3], Hans Joachim Ruscheweyh[4], Richard P. Darveau[5], Sarah Menz[1, 12], Alexandra Wittmann[1, 12], Patrick Adam[6], Bärbel Stecher[7, 12], Andreas Kulik[8], Daniel Huson[4], Ingo B. Autenrieth[1, 12] and Julia-Stefanie Frick[1, 12*]

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Background: The intestinal microbiota is crucial for shaping mucosal immunity and in inflammatory bowel diseases (IBD) the mucosal immune system interacts inappropriately with the intestinal microbiota. We showed for the first time that the composition of the intestinal microbiota results in different endotoxicity of the intestinal microbiota and that differential endotoxicity has a crucial impact on IBD development. **Methods:** We colonized Tcell-transferred Rag1$^{-/-}$ mice with two different types of complex intestinal microbiota characterized by 16S rDNA amplicon sequencing. **Results:** (i) Endo^{lo}-microbiota defined by low proportion of Enterobacteriaceae but high proportion of Bacteroidetes and exhibiting low entotoxicity resulted in mucosal immune homoeostasis, while (ii) the high endotoxic Endohimicrobiota consisting of high proportion of *Enterobacteriaceae* but low proportion of *Bacteroidetes* resulted in T_H1/T_H17-driven colitis. We hypothesized that colitogenicity of Endo^{hi}-microbiota might be related to the higher endotoxic activity of lipopolysaccharide (LPS) from *Enterobacteriaceae* compared to LPS from *Bacteroidetes*. Administration of *E. coli* JM83 (wildtype LPS) or *E. coli* +htrB$_{PG}$ (mutated LPS; lower endotoxicity like LPS of *Bacteroidetes*) or LPS isolated from these strains to mice resulted in exacerbation or prevention of colitis, respectively. **Conclusion:** Depending on the endotoxicity of their LPS commensals may favour immune stimulation resulting in immune homoeostasis or colitis. This principle might aid the design of novel probiotics that can be employed in the therapy of human inflammatory bowel disease.

Different influence of gut commensal bacteria on IBD

Anna Lange[1] , Sina Beier[1], Daniel Huson[2], Stefan Henz[3], Klaus Hantke[4], Christa Lanz[3], Ingo B. Autenrieth[1] and Julia-Stefanie Frick[1]

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The gut microbiota plays an important role in development of inflammatory bowel diseases (IBD). The commensal bacterium *E. coli mpk* can cause colitis in IL-2$^{-/-}$ mice due to a yet unknown mechanism. In contrast *B. vulgatus mpk* – another gut commensal – prevents induction of colitis by *E. coli mpk* in IL-2$^{-/-}$ mice. To identify potential inflammatory or anti-inflammatory bacterial compounds we performed whole genome sequencing of both microbes.

E. coli IBD isolates are reported to represent a heterogeneous population and it is not possible to screen for a specific IBD marker gene. But *E. coli mpk* genome harbours a set of common genes shared with other IBD-related *E. coli* isolates like genes for adhesion and biofilm formation, propanediol utilization, and haemolysis. Furthermore *E. coli mpk* encodes a complete operon for a functional type VI secretion system (T6SS). The system is particularly interesting as it can be involved either in interspecies competition or in mediating a pathogenic or symbiotic relationship with the host. T6SS knockout strains should be used to dissect its role during development of colitis. The genome of *B. vulgatus mpk* harbours a large number of genes related to polysaccharide metabolism, different LPS clusters, and sensory proteins.Here we give a first overview of the factors of commensal bacteria that might induce or prevent colitis in genetically predisposed hosts.

Characterization of a non-ribosomally synthesized peptide antibiotic from *Staphylococcus lugdunensis*

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[2]Institute of Organic Chemistry, University of Tübingen, Germany,
[3]Department of Microbial Genetics, University of Tübingen, Germany,
[4]Department of Microbiology/Biotechnology, University of Tübingen, Germany

Globally infectious diseases are the second-leading cause of death. A successful treatment of infectious diseases is threatened by the emergence and dissemination of antibiotic resistance amongst pathogenic bacteria, which increases continuously. This alarming development led to the urgent need of developing new antimicrobial drugs. Antibiotics resemble a part of the microbial defense system as they play an important role in competition between bacteria inhabiting the same natural system. They are vastly spread and highly abundant amongst bacteria, which makes them still attractive candidates for drug development. *Staphylococcus aureus*, one of the most successful and adaptable pathogens, is known to be a frequent colonizer of the human nose. A relationship between S. aureus nasal carriage and infection could already be shown by C. von Eiff (N Engl J Med, 2001). Due to early upcoming resistances, especially MRSA, nasal strains that coexist with S. aureus were of minor interest for researches. In this work, we wanted to investigate if there is competition between bacteria inhabiting natural systems like the nose, and if this competition leads to the production of antimicrobial substances. Therefore, single strains were isolated from the nose and tested for the production of antimicrobial substances. Among them was a *Staphylococcus lugdunensis* strain, which showed an inhibitory effect against *S. aureus*. Based on the transposon mutant *S. lugdunensis* M1, lacking its antimicrobial activity, the structure of a 30 kB operon encoding several structural and regulatory genes as well as potential ABC transporters was determined by PCR amplification and sequencing of the corresponding products. This enabled us to relate the antimicrobial effect to the production of a non-ribosomally synthesized peptide antibiotic, a group of antimicrobial peptides, which hasn't been described yet for staphylococci. For structure elucidation by NMR, this compound was purified by extraction, size exclusion chromatography and PHPLC from culture supernatants of the genetically modified strain *S. lugdunensis* Xyl, which enables a xylose-inducible peptide production.

Role of the SOS response in the development of heterogeneity within *Staphylococcus aureus* populations

Shilpa E. George, Wiebke Schröder, Christiane Wolz

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Bacteria respond to stressful conditions by a plethora of adaptive mechanisms such as activation of alternative sigma factors, stringent response and the SOS response. The SOS response is induced upon DNA-double strand breaks which then leads to the activation of the RecA protein. RecA is not only important for DNA repair but also results in the auto cleavage of the transcriptional repressor LexA thus activating the SOS response genes. This involves the induction of error-prone polymerases which results in increased mutation frequency. Several antibiotics can induce the SOS response at sub-inhibitory concentrations which accelerates the emergence of antibiotic resistance due to higher mutation rates.

In this study, we find that in *Staphylococcus aureus*, non-hemolytic variants develop rapidly over time in response to sub-inhibitory concentrations of SOS inducing antibiotics. These variants were found to be defective in function of the "quorum sensing"system *agr*. Further, agr defective strains were shown to have a fitness advantage over the wild type. We created *lexA* and *recA* mutants in order to assess the role of the SOS response in the development of this phenotype. The non-cleavable *lexA* mutant was still capable of developing this phenotype over several subcultures. However, fewer variants accumulate in the *recA*- mutant background. The maintenance of such diversity can be of adaptive value, increasing the fitness of the bacterial community as a whole.

Investigation of nitrogen regulation by a putative $P_{II\text{-}like}$ protein in *Staphylococcus aureus*

Ali Coskun[1], Karl Forchhammer[2], Friedrich Götz[1]

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[2]Interfakultäres Institut für Mikrobiologie und Infektionsmedizin, Department of Organismic Interactions, Tuebingen, Germany

P_{II} proteins are one of the most known families of signal transduction proteins in bacteria, archaea, and plants. They coordinate and regulate many aspects of nitrogen metabolism by interacting with the activities of enzymes, transcription factors, and membrane transport proteins. They have also been proposed that their functions may extend to the coordination of nitrogen and carbon metabolism and to sensing of cellular energy status. The primary mode of signal perception appears to be almost universal and involves the binding of the effector molecules 2-oxoglutarate (2-OG) and ATP/ADP within the lateral intersubunit clefts.

A secondary mode of signal perception, which is less conserved, involves covalent modification of a residue within the T-loop. P_{II} proteins can regulate the glutamine synthetase (GS) activity by sensing the nitrogen state of cells. The nitrogen state of the cell also affects transcription of the gene that encodes GS, *glnA*. In the genome of *Staphylococcus aureus* NCTC8325, SAOUHSC_00452 gene encoding a protein which has a similarity to nitrogen regulatory $P_{II\text{-}like}$ protein and it was annotated as a hypothetical protein. In this study, we generated a putative $P_{II\text{-}like}$ deletion mutant in *Staphylococcus aureus* NCTC8325 and studied its function by comparative phenotypic analysis. Deletion of the putative $P_{II\text{-}like}$ gene resulted in severe impairment during the exponential phase of growth in nitrogen deficient minimal media and nitrogen excess rich medias. Also, we observed similar growth delays in the presence of nitrate during anaerobic growth. Analysis of growth with various nitrogen sources showed no significant differences. In addition, nitrite accumulation in medium between wild type and $\Delta P_{II\text{-}like}$ strain was at the same rate. Otherwise, $\Delta P_{II\text{-}like}$ showed higher glutamine synthetase activity compared the wild type strain. This result suggest that high GS activity might be cause of poor growth in $\Delta P_{II\text{-}like}$ during the exponentially phase. Also, $\Delta P_{II\text{-}like}$ led to a more biofilm formation phenotype than wild type strain. This result indicate that the possible roles of $P_{II\text{-}like}$ protein in the central metabolic process of cells need to be discovered.

The Influence of Cell-Cell Interactions on Cellular Decision Making

Simon van Vliet, Martin Ackermann

ETH Zurich & Eawag, Switzerland

Clonal microbial populations growing in a homogeneous environment often show large phenotypic variation. This heterogeneity is often attributed to stochastic gene expression, however recently it has been found that some of this variation is a consequence of deterministic factors like epigenetic inheritance or the population context of a cell. Most work so far has focused on explaining phenotypic variation by using processes working at the level of individual cells. However, for sessile bacteria there is a potential for neighboring cells to influence each other's phenotypes. These interactions can be direct, e.g. mediated by quorum sensing or cell signaling, or passive as a cells phenotype will affect its microenvironment, which subsequently influences the cellular- decision making of its neighbors.

Here we address the question to what extend interactions between cells contribute to phenotypic variation in bacterial colonies. Using time-lapse fluorescence microscopy we followed the expression level of the bacteriocin Colicin Ib in micro-colonies of *Salmonella typhimurium* and *Escherichia coli*. Visual inspection of colonies showed that neighboring cells often have similar phenotypes. We developed a statistical method to test if this apparent similarity between neighboring cells is caused by cellcell interactions or if it can be explained by the, on average, higher relatedness of neighboring cells. Using a computational model we validated this statistical method.

Preliminary results show that neighboring cells are significantly more similar than can be expected based on their relatedness alone, suggesting that Colicin Ib expression levels are influenced by cell-cell interactions.

Genome-wide approaches towards understanding fitness determinants of *Pseudomonas aeruginosa* in the environment and during infection

Stephen Lory[1], Deborah Yoder-Himes[1], David Skurnik[2], Damien Roux[2], Vincent Cattoir[1], Gerald Pier[2]

[1]Harvard Medical School, Boston, USA, Channing Laboratory,
[2]Brigham and Women's Hospital, Boston, USA

Publication of the first complete genome sequence of an organism (a bacterium) in 1995 profoundly affected all fields of life sciences, particularly microbiology. Availability of complete annotated genome sequences of hundreds of bacterial species provide new tools and resources for better understanding all aspects of bacterial life, ranging from basic metabolism, ecology, to evolution. Some of the surprising findings were uncovered from the application of whole genome sequencing and related approaches of functional genomics, altering our understanding of the biology of the opportunistic pathogen *Pseudomonas aeruginosa*. Considering the relatively large coding capacity of its genome and the variety of environmental niches this organism can occupy, it is not surprising that *P. aeruginosa* have evolved a complex regulatory network, consisting of transcriptional and post-transcriptional regulatory mechanisms that allow these bacteria to thrive in widely differing natural environments including infected hosts. Specific examples of unusual virulence vectors and host-defense evasive strategies will be presented. Implications of findings arising generated by new sequencing technologies in defining function of uncharacterized genes and their products will be also discussed.

What small RNAs can do for *Pseudomonas* and related bacteria

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When bacteria adapt to changing environmental conditions by adjusting transcription, they basically have two options: Either transcriptional expression of structural genes is controlled directly or synthesis of regulatory small RNAs (sRNAs) is controlled, often via two-component systems that can sense environmental changes. In turn, many such sRNAs ensure control of gene expression at the level of translation initiation. Whereas in enteric bacteria sRNAs appear to be involved mainly in the management of stress, in pseudomonads several sRNAs have been characterized that operate important metabolic switches. Three examples in *Pseudomonas* spp. will be discussed. (i) The Gac/Rsm system is a major switch for the formation of extracellular factors and secondary metabolites. In the plant-beneficial rhizobacterium *Pseudomonas protegens*, this switch not only depends on the three GacS/GacA-dependent sRNAs RsmX, RsmY and RsmZ, but also on the concentration of the response regulator GacA itself, which was found to be under tight control by Lon protease. (ii) The Cbr/Crc pathway of *Pseudomonas aeruginosa* selects the energetically most suitable C and N sources by catabolite repression of degradation functions for less favourable substrates. While an initial model postulated that the CbrA/CbrB-dependent sRNA CrcZ would bind and thereby titrate the catabolite repression control protein Crc, recent data from several laboratories indicate that CrcZ binds the RNA-binding protein Hfq, which turns out to be the master regulator of catabolite repression in *P. aeruginosa*. (iii) The NtrB/NtrC two-component system, which is known to prioritize N sources in many bacterial species, also elaborates an sRNA, NrsZ, in *P. aeruginosa*. By a base-pairing mechanism, NrsZ controls translation of target genes in this opportunistic pathogen and thereby impacts on vital functions beyond N metabolism.

Post-translational regulation of alginate export by an oxygen sensor in *Pseudomonas aeruginosa*

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To respond to environmental oxygen limitation, many facultative anaerobic bacteria use elaborate oxygen sensing mechanisms coupled to signalling cascades which allow optimizing a microaeorbic/anaerobic lifestyle. *Pseudomonas aeruginosa* up-regulates production of the exopolysaccharide alginate under oxygen limitation leading to biofilm formation and pathogen persistence in airways of patients with cystic fibrosis (CF). The molecular mechanisms leading to this phenotypic change are largely unknown. Here we describe a three component system (OraB/OraA/SadC) in P. aeruginosa which controls alginate production and mucoidy via the second messenger cyclic bis-(3'-5')-diguanylate monophosphate (c-di-GMP). We show that in the presence of oxygen purified OraA inhibits c-di-GMP production of the purified cytosolic fragment of the diguanylate cyclase SadC, while purified OraB re-activates SadC to produce c-di-GMP. To elucidate the reaction mechanism, we synthesized a 20 amino acid peptide, containing the structural domain of SadC, GGEEF. Incubation of the peptide with the hydroxylase OraA resulted in hydroxylation of a proline residue (HO-P), adjacent to GGEEF. Mutations in *sadC* or *oraB* in strain PAO1 resulted in a profound loss of alginate synthesis, whereas mutations in oraA allowed constitutive alginate synthesis under aerobic and anaerobic growth conditions. This novel post-translational regulatory mechanism links oxygen sensing with c-di-GMP-coupled signal-transduction and exopolysaccharide production in *P. aeruginosa*.

Ion pumps in *Staphylococcus aureus* – how do staphylococci generate membrane potential?

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In many aerobic and facultative aerobic microorganisms the first complex of the respiratory chain is the NADH-quinone oxidoreductase. In *Escherichia coli* the NADH-dehydrogenase complex I is well studied and is composed of more than 12 proteins and genes (*nuoA-N*). In *Staphylococcus* a corresponding gene cluster is absent and therefore the question remains how the respiratory proton/ion translocation is mediated in staphylococci.

We have identified a hypothetical protein in *Staphylococcus aureus* showing relatively high sequence similarity to a proton-translocating subunit of NADH-quinone oxidoreductase in *E. coli* and many other prokaryotes. The hypothetical homolog in *S. aureus* is a membrane protein and apparently organized in an operon comprising three genes. The first gene and the complete operon were deleted and phenotypically analyzed. Both deletions showed a severe growth defect, a small colony variant phenotype, decreased membrane potential and decreased oxygen consumption rates. The results suggest that this operon plays a crucial role in energy metabolism and cellular fitness.

The Iron Uptake and Distribution System in *Bacillus subtilis*

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Iron is a crucial nutrient for almost all living organisms on earth. Due to its standard redox potential it functions as an excellent co-factor for the catalysis of redox reactions. In most bacteria iron is required for the survival and virulence and amongst other it is involved in the electron transfer, cell respiration and superoxide metabolism. But despite its high abundance on earth it remains poorly available due to the low solubility of ferric iron (Fe^{3+}) and the spontaneous oxidation of ferrous iron (Fe^{2+}) under physiological aerobic conditions.

Many organisms acquire iron by secreting low molecular weight compounds called siderophores, which sequester ferric iron in high affinities. The ferri-siderophore complexes are imported into the cell by a variety of iron regulated ABC- transporter systems(1,2). For *Bacillus subtilis* we could show the uptake of the siderophores bacillibactin and the xenosiderophore enterobactin by the FeuABC machinery(2,3). Once the ferri-siderophores is imported it is either hydrolysed, as we could show for the bacillibactin hydrolase BesA(3), or the iron-siderophore binding gets weakend by reduction of the iron. In *E. coli* we could demonstrate the reductive release of iron from enterobactin catalyzed by the siderophore binding protein YqjB(4). Recently, our group published a siderophore independent uptake system in *B. subtilis* capable of importing ferrous and ferric iron(5).

The further intracellular transport of the iron to its destination remains elusive, but it could be demonstrated in *B. subtilis* that the frataxin ortholog Fra is able to bind iron and deliver it to the iron-sulfur cluster machinery SufS-SufU(6). A physical interaction of Fra with the Fe-S cluster assembly machinery has been demonstrated(7,8)[], but the role as an iron carrier remains controversial. We could provide evidence that frataxin is also able to deliver iron to the iron chelatase HemH in the heme maturation pathway(9). We therefore assume that frataxin plays a central role in the iron distribution network in *Bacillus subtilis*. Our current work focusses on the understanding of how iron gets distributed in *B. subtilis* and how Fra is involved in the iron channeling pathways.

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Frataxin acts as an essential iron carrier in the heme maturation pathway of *Bacillus subtilis*

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Frataxin is a small protein with a characteristic α-β sandwich fold that is conserved among prokaryotes and eukaryotes. It is suggested to act as a regulatory component as well as an iron chaperone in different cellular pathways including iron-sulfur cluster (ISC) and heme biogenesis. In this study we investigated the role of the *B. subtilis* frataxin homolog Fra in heme maturation and present for the first time an intracellular trafficking pathway that essentially relies on frataxin-dependent iron transfer. By using the recombinant proteins of this pathway we could show that Fra is able to serve as an acceptor for ferrous iron in interaction with the ferric iron reductase EfeR, which catalyzes the first step of cytosolic iron assimilation in association with the iron-inducible NADPH:ferredoxin oxidoreductase FfoR. Further downstream, iron-charged frataxin is able to act as a ferrous iron donor during the final step of heme *b* assembly catalyzed by the ferrochelatase HemH, which is a prerequisite for the acquisition of heme by cellular target proteins such as the nitric oxide synthase bsNos. Complementary *in vivo* studies with frataxin-deficient cells confirmed that frataxin serves as a key component for iron charging and subsequent targeting of the released heme cofactor. Hence, the iron carrier frataxin plays a central role in the cellular physiology of *B. subtilis*.

Insights into lysolipin biosynthesis regulation

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Streptomyces tendae Tü 4042 produces the aromatic polyketide antibiotic lysolipin (1). Lysolipin is highly active against Gram-positive bacteria (MIC < 10 ng/ml) and targets a yet unidentified component in the bacterial cell envelope (2). It also shows strong cytotoxic effects on eucaryotic cells and might therefore be involved in active defense against bacteria as well as higher organisms.

The lysolipin biosynthetic gene cluster was identified, sequenced and annotated. It encodes 44 genes involved in biosynthesis, regulation and export of the antibiotic. All genes necessary for lysolipin production are located on a 43 kb cosmid. This was shown by successful expression of the lysolipin gene cluster in the heterologous host *Streptomyces albus.* LC-MS and HPLC analysis showed yields of the antibiotic comparable to these of the wild type strain (3).

We have identified five regulatory genes (*llp*RI-V) which may be involved in transferring environmental signals to initiate the onset of lysolipin production. LlpRIV has high sequence similarity with *Streptomyces* Antibiotic Regulatory Proteins (SARPs), which usually act as activators. LlpRII and LlpRIII are activators of the TenA family. LlpRI shows similarity to putative PadR-type transcriptional repressors. The sequence of LlpRV resembles a putative DNA-binding protein of *Streptomyces coelicolor* A3 (2). The five regulatory genes were expressed as His-tag fusion proteins in the heterologous host E. coli Rosetta 2 (DE3) pLysS using the vector pET30Ek/LIC. With these His-tag fusion proteins bandshift assays with putative promoter regions can be used to further analyse the role of these five putative regulators.

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Metabolic changes in *Synechocystis PCC6803* upon nitrogen starvation: Excess NADPH sustains Polyhydroxybutyrate accumulation

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Polyhydroxybutyrate (PHB) is a common carbon storage polymer among heterotrophic bacteria. It is also accumulated in some photoautotrophic cyanobacteria; however, the knowledge of how PHB accumulation is regulated in this group is limited. PHB synthesis in *Synechocystis* sp. PCC 6803 is initiated once macronutrients like phosphorus or nitrogen are limiting. We have previously reported a mutation in the gene *sll0783* that impairs PHB accumulation in this cyanobacterium upon nitrogen starvation. In this study we present data which explain the observed phenotype. We investigated differences in intracellular localization of PHB synthase, metabolism, and the NADPH pool between wild type and mutant. Localization of PHB synthase was not impaired in the *sll0783* mutant. Metabolome analysis revealed a difference in sorbitol levels, indicating a more oxidizing intracellular environment than in the wild type. We confirmed this by directly measuring the NADPH/NADP ratio and by altering the intracellular redox state of wild type and *sll0783* mutant. We were able to physiologically complement the mutant phenotype of diminished PHB synthase activity by making the intracellular environment more reducing. Our data illustrate that the NADPH pool is an important factor for regulation of PHB biosynthesis and metabolism, which is also of interest for potential biotechnological applications.

Identification of a novel player in cyanobacterial PHB metabolism

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Several cyanobacterial strains are able to produce the carbon storage polymer polyhydroxybutyrate (PHB) but, so far, no extensive study was performed to identify PHB granule associated proteins in cyanobacteria. Accumulation of PHB in cyanobacteria takes place under different starvation conditions, such as nitrogen or phosphate starvation. Based on protein analysis of PHB granules and genomic comparison, we identified a putative phasin in the cyanobacterium *Synechocystis sp.* PCC 6803. To investigate the role of the protein, it was fused to the green fluorescence protein GFP. Thereafter, we checked the abundance of the hypothetical phasin via western blot analysis under different conditions, such as, exponential growth, nitrogen starvation and further more. By fluorescence microscopy, we showed co-localization of the potential phasin and the PHB granules. Furthermore, a knockout mutant of the potential phasin was constructed. This mutant strain revealed a significant difference in the amount and the shape of the native PHB granules compared to the wildtyp strain, in accord with its predicted function. Thus, we assume that we identified the first cyanobacterial phasin, termed „PhaP". Its role in PHB metabolism is similar to that of so far characterized phasins in determining the surface-area-to-volume ratio of PHB granules.

Isolation and characterization of an unknown toxin from *Synechococcus elongatus PCC7942*

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The cyanobacterial production of bioactive metabolites is a poorly understood and explored field of the Natural Product Research. Although a high amount of bioactive metabolites from Cyanobacteria were characterized in the past years, the bulk remains to be explored. Known cyanobacterial metabolites show antiviral, anti-tumour, antibacterial and anti-HIV activities.

This work shall contribute to the knowledge of Cyanobaceria as a valuable source of bioactive molecules. It describes the isolation of a compound secreted by *Synechococcus elongatus* PCC7942 with bioactive characteristics against various Cyanobacteria. Due to the high polarity of the metabolite, several purification steps, such as Size-Exclusion-Chromatography, Thinlayer-Chromatography and HPLC-DAD-ELSD Chromatography after derivatization were established. First results of LC/MS- and GC/MS-Chromatography and H^1-NMR target a sugar-derivative to be responsible for the bioactivity.

CRISPR/Cas with a grain of salt

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We are investigating the recently discovered prokaryotic immune system, the CRISPR/Cas system, in the halophilic archaeon *Haloferax volcanii*. The CRISPR/Cas system (CRISPR: clustered regularly interspaced short palindromic repeats, Cas: CRISPR – associated) is an adaptive and heritable resistance mechanism against foreign genetic elements. The CRISPR-Cas system consists of clusters of repetitive chromosomal DNA in which short palindromic DNA repeats are separated by spacers, the latter being sequences derived from the invader. In addition, a set of proteins, the Cas proteins, is involved. The defence mechanism progresses in three stages: (1) adaptation, the acquisition of new spacers; (2) expression, the production of mature CRISPR-RNAs, and (3) interference, the degradation of invader-DNA.

We are investigating the CRISPR/Cas system in the halophilic archaeon *Haloferax volcanii*. *H. volcanii* is an archaeal model organism that requires about 2.1 M NaCl for optimal growth. The genome is sequenced and it is one of the few archaeal organisms where genetic systems are available. We are analysing the function of one of the key players of the system, the endonuclease Cas6.

The impact of secondary metabolites on the versatile lifestyle of the symbiotic cyanobacterium *Nostoc punctiforme*

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The strain *Nostoc punctiforme* is a filamentous, nitrogen-fixing cyanobacterium, whose vegetative cells can mature in four different directions. *N. punctiforme* has a very broad symbiotic capacity and interacts with diverse plant species. The strain ATCC29133 is capable of producing a variety of secondary metabolites, including some of the nonribosomal peptide and polyketide classes. Our investigations suggest a role of different metabolites in the orchestration of cellular differentiation events and communication with symbiotic hosts. Insertional mutagenesis of a gene of the polyketide synthase gene cluster *pks2* led to the accumulation of short filaments carrying mostly terminal heterocysts under diazotrophic conditions. The mutant has a strong tendency to form biofilms on solid surfaces as well as in liquid culture. The *pks2*$^-$ strain keeps forming hormogonia over the entire growth curve and shows an early onset of akinete formation. Growth of the mutant cells in the neighbourhood of wild-type cells on plates led to a reciprocal influence and a partial reconstruction of wild-type and mutant phenotype respectively. Here, we report, how further secondary metabolites are interfering with these changes in cellular differentiation and how they are responding to the interaction with the symbiotic partner *W*. We have used MALDI-Imaging to visualize the metabolites as well as mutagenesis and expression analysis to assess their guiding role role in the life cycle. A focus will be given to the group of nonribosomal nostopeptolides that are predominant secreted factors in *N. punctiforme*.

Phenotypic heterogeneity in bacterial metabolic activities for growth during fluctuating nutrient supply

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Genetically identical cells that live in a homogeneous environment often show substantial variation in their biological traits; such variation is called phenotypic heterogeneity. The level of phenotypic heterogeneity can evolve, because it has a genetic basis and it provides - as suggested by theoretical studies - a population of genotypes with an efficient way to respond to uncertain environments. However, it is until now not known if phenotypic heterogeneity is relevant for bacterial metabolic activities that contribute to biogeochemical cycles. In addition, direct experimental evidence that such metabolic heterogeneity provides individuals with a growth advantage in fluctuating environments is missing on a single-cell level. We use a combination of single-molecule mRNA FISH and nanometer-scale secondary ion mass spectrometry (nanoSIMS) to experimentally investigate how phenotypic heterogeneity affects bacterial metabolic activity and growth under fluctuating environmental conditions. NanoSIMS directly measures the metabolic activity of single cells based on the assimilation of stable isotopes from a labeled substrate. Specifically, we investigate phenotypic heterogeneity in N_2-fixation in the model organism Klebsiella oxytoca under fluctuating NH_4^+ concentrations. We show that nitrogenase (key enzyme for N_2-fixation) expression is heterogeneous if cells are grown under limited NH_4^+ supply. Heterogeneous populations retain a positive growth rate when exposed to a sudden switch from limited NH_4^+ supply to NH_4^+-deplete conditions. Using a nanoSIMS-based pulse-chase approach we demonstrate the fittniess benefits for nitrogenase-active cells under fluctuating NH_4^+ supply. We hypothesize that phenotypic heterogeneity in metabolic activity is a microbial bet-hedging strategy during nutrient fluctuations

The secondary metabolite iromycin as regulator and quorum sensing molecule

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Iromycin A (**1**) is a member of a new family of rare α-pyridone metabolites(1) which are produced by the Streptomcete strain Gö Dra 17 among other metabolites like diketopiperazines. Ratios of the metabolite production, e.g. mg/L-scale, are strongly dependent on the different, optimized cultivation media.
The isolation and structure elucidation revealed an N-heterocyclic core structure with two unusual side chains. Though the branched C_{10} side chain might imply isoprenoid origin, biogenetic investigations indicate a polyketide biosynthesis.
In biological test systems iromycin A appears as a very weak antibacterial agent, however, as a unique selective inhibitor of nNOS and eNOS (neuronal, resp. endothelial nitric oxide synthase) which can differentiate among them for the first time.

To investigate and reveal the iromycin biosynthetic gene cluster and the regulation system whole genome sequencing was performed. Remaining gaps within the hypothetic iromycin biosynthetic gene cluster have been closed via PCR.(2) Applying a gfp construct the conjugation protocol(3) for this wildtype strain has been established. Currently, a partial deletion of the hypothetic biosynthetic gene cluster by double cross over is envisaged to proof the presumable biosynthetic gene cluster which we assigned to iromycin. By constructing further knock out mutants it should then be possible to reveal the biosynthesis of iromycin. We are especially interested in the regulation network among the yet detected metabolites and their importance for the survival of the bacterial producer strain.

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Profound Implications of Microcystin Production in *Microcystis aeruginosa*

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Mass developments of toxic cyanobacteria can have severe impact on drinking water supply and the recreational value of lakes. Microcystin is a nonribosomally synthesized- and highly toxic peptide produced by the bloom-forming cyanobacterium *Microcystis aeruginosa*. Despite many years of research the question about the primary biological role of microcystin has not been answered yet. Recent discoveries imply direct physiological functions of microcystin. The interaction of the secondary metabolite with primary cell metabolism might be realized via binding to specific cysteine residues of proteins *in vivo*(1). Utilization of a new immunological method revealed that exposition of *Microcystis* cells to high light has a major influence on the amount of protein bound microcystin. Up to 60 % of total cellular microcystin can be found in that otherwise inaccessible fraction(2). The major microcystin binding partner is the large RubisCO subunit. The modification was shown to render the protein less susceptible towards proteolysis. Additionally, first data from a metabolomics experiment indicate involvement of microcystin in tuning major carbon pathways. Loss of microcystin production affects the Calvin-Benson cycle, photorespiration and the tricarboxylic acid cycle. In conclusion, the data suggest a light dependent protein-modulating role of microcystin with profound physiological consequences for the producing organism.

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Archaeal motility

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Most archaea posses a motility structure, called the archaellum that is mainly used for swimming, but can also play a role in adherence to surfaces. Although the archaellum functionally resembles the bacterial flagellum, its structure and assembly is reminiscent of bacterial type IV pili.
We use the crenarchaeal archaellum of S*ulfolobus acidocaldarius* as a model system to understand its assembly, subunit interactions and how it can rotate. The crenarchaeal archaellum is comprised of only seven subunits and we use biochemical and genetic approaches to understand their function in archaellum assembly and rotation. Biophysical studies like tethered motion particle analysis and optical tweezer experiments are being used to understand the mode of rotation of the archaellum and will be presented. Additionally, we have started to uncover the network that is involved in the regulation of archaellum biosynthesis.

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

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The *N*-glycosylation process is conserved across all three domains of life. In contrast to Bacteria N-glycosylation occurs in almost all Archaea, which exhibit a high variety on dolichyl pyrophosphate linked *N*-glycans, in terms of structure and composition. However, the *N*-glycosylation process in the ancient archaeal kingdom, the crenarchaeota is still unknown. Here we will report the first results elucidating the *N*-glycosylation pathway in the thermoacidophilic archaeon *Sulfolobus acidocaldarius*. The S-layer protein SlaA, used as a reporter glycoprotein, possesses a remarkably high glycosylation density, averaging one site for each stretch of 30–40 residues. Each of the glycosylation sites observed was shown to be modified with a heterogeneous family of glycans, with the largest composed of a tribranched hexasaccharide (Glc-QuiS)-(Man)-(Man)-$GlcNAc_2$, which contain a chitobiose core reminiscent of the eukaryal *N*-glycans. A marker less deletion mutant of the gene coding for UDP-Sulfoquinovose revealed a reduced *N*-linked glycan on the S-layer composed of only the trisaccharid Man_1-$GlcNAc_2$. Furthermore we could identify the first crenarchaeal GTase transferring the last sugar onto the dolichyl-pyrophosphate linked *N*-glycan. Defects in the *N*-glycosylation results in a severe reduction of growth at elevated salinities, a strong reduction of motility, and reduction of anti microbial behavior, which underlines the importance of this post translation modification for the survival of this microorganism.

CRISPR/Cas with a grain of salt

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To defend themselves against foreign genetic elements like viruses and plasmids, prokaryotes have developed a plethora of strategies. A recently discovered way to fend off invaders is the CRISPR/Cas system (clustered regularly interspaced short palindromic repeats/ CRISPR associated). The CRISPR/Cas system provides adaptive and heritable immunity against foreign genetic elements. Essential for the function of this system are the Cas proteins and the CRISPR RNAs. The latter is transcribed from the CRISPR locus and consists of short sequence repeats in between which spacer sequences are located. The spacer sequences are derived from previous invaders, which have been successfully destroyed. Their DNA has been degraded and a piece of it has been selected to be integrated as a new spacer into the CRISPR locus. Thus the CRISPR locus is a memory of previous attacks, from which the cell has survived. Since the information about the invader is stored in the genome, the whole system is hereditary. Besides the CRISPR RNA, the other key players are the Cas proteins. Although this system is widespread and diverse with many subtypes, only few species have been investigated to elucidate the precise mechanisms for the defence of viruses or plasmids. We are investigating the CRISPR/Cas system in *Haloferax volcanii*.

Host phylogeny and microenvironment are major drivers of methanogenic community structure in arthropod guts

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Termites, cockroaches, scarab beetle larvae, and millipedes are the only arthropods known to harbor methanogens in their intestinal tracts. Particularly the termites, whose methane emission rates rank second only after the ruminants, contribute substantially to the global budget of this greenhouse gas. However, only little is known about the diversity of methanogens and their community structure in their sometimes highly compartmented guts. We analyzed the archaeal communities in a wide range of host species, using a combination of cloning and pyrotag sequencing of the 16S rRNA gene. The assemblages of methanogenic archaea in different hosts showed distinct phylogenetic patterns. Highest diversity was found in soil-feeding termites, which are the highest methane emitters and harbor methanogens of several orders, including the recently discovered ‚Methanoplasmatales'. To identify the environmental drivers responsible for the differences in community structure observed between the consecutive gut compartments and the functional roles of the different populations, we investigated the stimulation of methanogenesis by the addition of potential substrates. Our results indicate that the structure of the methanogenic communities in arthropod guts are shaped by a combination of host phylogeny and exogenous factors, including pH, oxygen status, and the availability of methanogenic substrates.

"Methanoplasmatales" - diversity, ultrastructure, and comparative genomics of the 7th order of methanogens

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We recently showed that a deep-branching lineage of Euryarchaeota distantly related to the *Thermoplasmatales* represents the seventh order of methanogens. The congruent phylogenies of 16S rRNA and *mcrA* gene trees indicate the presence of "Methanoplasmatales" in diverse environments, including soil, marine habitats, and the intestinal tracts of arthropods and mammals. We obtained highly enriched cultures of strains MpT1 and MpM2 from the gut of termites and millipedes. Ultrastructural analysis documented the absence of a cell wall and the presence of an unusual two-layered membrane system, which may be characteristic for all members of the order. The genome of strain MpT1 was sequenced (1.49 Mb). It contains all genes required for methanogenesis from methanol with H_2 as electron donor, whereas all other genes of the pathway reducing CO_2 to the methyl level (or the oxidation of the latter to CO_2) are lacking, which explains why strain MpT1 can neither reduce CO_2 to CH_4 nor disproportionate methanol to CO_2 and CH_4. We compared our results to the draft genomes of the first isolate of "Methanoplasmatales" and other highly enriched strains from human feces.

Fe(III) mineral formation by iron(II)-oxidizing bacteria – consequences on microbial survival and activity

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The two most important redox states of iron in the environment are Fe(II) [ferrous iron] and Fe(III) [ferric iron]. Redox transformation of iron, including Fe(II) oxidation and Fe(III) reduction, is used by many microorganisms to produce energy and to grow and leads either to dissolution, transformation or precipitation of iron minerals. At neutral pH, oxidation of dissolved ferrous iron [Fe(II)] and precipitation of poorly soluble Fe(III) minerals can be catalyzed by microaerophilic, nitrate-reducing and even phototrophic microorganisms. This presentation will show results regarding physiology, ecology and environmental implications of microbial Fe(II) oxidation and will discuss potential consequences of microbial mineral formation on survival of the microorganisms. The focus will be on microaerophilic Fe(II)-oxidizing bacteria that thrive in gradients of ferrous iron and oxygen (e.g. surface-near lake sediments and rice paddy soils), on nitrate-reducing Fe(II)-oxidizers and on phototrophic Fe(II)-oxidizing bacteria that we suggested to have contributed to the deposition of ancient rock deposits, so-called Banded Iron Formation, billions of years ago.

Iron(III) minerals as a protection mechanism from UV radiation and/or desiccation by neutrophilic iron(II)-oxidizing bacteria

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Ferrous iron is relatively soluble at neutral pH but the product of microbial Fe(II) oxidation, i.e. ferric iron, is poorly soluble. Hence microbial Fe(II) oxidation at neutral pH leads to precipitation of Fe(III), typically in form of Fe(III) (oxyhydr)oxides. Due to their positive surface charge, the Fe(III) minerals are expected to interact with negatively charged cell surfaces. In case of *Acidovorax* sp. strain BoFeN1, a mixotrophic nitrate-reducing Fe(II)-oxidizer, the cells even become encrusted in Fe(III) minerals. In contrast, anoxygenic phototrophic Fe(II)-oxidizing bacteria, so called photoferrotrophs, do not become encrusted during Fe(II) oxidation but the cells are closely associated with the precipitated Fe(III) minerals (Fig.1).

Fe(III) minerals absorb UV light effectively, but most of them still transmit visible light, e.g. ferrihydrite ($Fe_{10}O_{14}(OH)_2$) (Bishop *et al.*, 2006). Consequently the encrustation of Fe(II)-oxidizing strains or close association with Fe(III) minerals in case of the photoferrotrophs could serve as a protection mechanism against UV light, which otherwise causes damages to the cells' DNA. Additionally, silicification of cells was hypothesized to be an effective protection against intermittent dehydration events (Phoenix & Konhauser, 2008); this could be true in a similar fashion for iron crusts. Thus, in this project we investigate if iron biomineralization and close association of cells with Fe(III) minerals serves as a UV light screen or/and a desiccation protection for Fe(II)-oxidizing bacteria.

Therefore, encrusted and non-encrusted cells of different Fe(II)-oxidizing bacteria are currently investigated. *Acidovorax* sp. BoFeN1 is grown with dissolved Fe(II), acetate (organic co-substrate), and NO_3^- (electron acceptor), which leads to encrusted cells. An iron-free control is grown on the same medium but without Fe(II), which leads to non-encrusted cells. *Rhodobacter ferrooxidans* strain SW2 and *Rhodopseudomonas palustris* strain TIE-1 are grown photoautotrophically either with Fe(II), leading to a close association of cells and Fe(III) minerals, or with H_2 as electron donor, which leads to a mineral free environment.

After exposure to UV light or dehydration of the bacterial cells, the cell viability is determined by quantifying colony forming units (CFU), microscopic dead/live staining and iron oxidation/nitrate reduction rates. To quantify biological effects of UV radiation different cellular indicators of oxidative stress (lipid peroxidation, DNA strand breakage, protein oxidation, generation of reactive oxygen species) are used. By comparison of the viability of cells with or without UV treatment we will be able to assess if Fe(III) minerals do protect Fe(II)-oxidizing bacteria from UV radiation or desiccation.

Two component systems involved in regulation of anaerobic hydrocarbon metabolism

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Aromatic hydrocarbons like benzene, toluene, ethylbenzene and xylenes (BTEX) are used in large amounts in industrial processes and therefore lead to widespread environmental pollution. Because of their high solubility in water, these compounds easily reach groundwater or deep layers of soil, where they encounter anoxic conditions. The genome-sequenced β-proteobacterium *Aromatoleum aromaticum* is capable to degrade the hydrocarbons toluene and ethylbenzene as well as different phenolic compounds anaerobically(1,2). The genes coding for the enzymes of anaerobic toluene metabolism are induced co-ordinately in the presence of toluene, whereas those coding for the enzymes of anaerobic ethylbenzene metabolism are induced sequentially in the presence of ethylbenzene and the intermediate acetophenone, respectively. Three operons coding for two-component regulatory systems were identified in the genome sequence of *A. aromaticum* as possible candidates for affecting the induction of all toluene-catabolic genes (*tdiSR*), the induction of ethylbenzene-catabolic genes by ethylbenzene (*ediSR*) and the induction of acetophenone-catabolic genes by acetophenone (*adiRS*). Our main aim is to clarify the mechanisms involved in discrimination of these very similar aromatic substrates.

The two-component regulatory systems AdiRS, EdiSR und TdiSR are composed of sensory histidine kinases and response regulators with considerable similarity to each other. All components are predicted to be soluble cytoplasmic proteins. In our studies we show that the *adiRS* operon is indeed involved in the acetophenone-dependent induction of gene expression. The function of these gene products was investigated by genetic and biochemical studies. Moreover, the predicted acetophenone-sensing histidine kinase (AdiS) and the corresponding response regulator (AdiR) were overproduced in *E. coli* and purified. First results on their biochemical properties will be shown.

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σ^{54} promoters as targets of substrate-specific induction of catabolic pathways

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The facultative anaerobic denitrifying bacterium *Aromatoleum aromaticum* is able to distinctively degrade hydrocarbons and phenolic compounds anaerobically. The anoxic degradation of the very similar compounds toluene. ethylbenzene, phenol, *p*-cresol and *p*-ethylphenol is separately regulated for each substrate. Genes encoding putative regulators (PdeR1 and PdeR2) were identified close to the operons containing the genes for the enzymes involved in anaerobic phenol and *p*-ethylphenol degradation, respectively. Both regulators belong to the XylR-like regulators, which are found in a variety of bacteria (e.g. *Escherichia coli, Pseudomonas putida, Klebsiella pneumoniae*) regulating the metabolism of a high diversity of substrates. Regulators of this group bind to specific consensus sequences positioned upstream of σ^{54} promoter regions. The specific promoter sequence contains located GG motif at position -24 and a GC motif at position -12 relative to the transcriptional start. The regulator proteins contain a helix-turn-helix type DNA binding site, which is linked to a domain involved in dimerization and ATPase activity. Furthermore, the regulators have a specific sensory domain to recognize the substrate (e.g. phenol). In the inactive state of the regulator, the sensor domain blocks the ATPase domain, whereas it changes its conformation in the substrate-bound state to activate the ATPase domain. The regulator binds to the DNA and initiate transcription of target genes of under ATP-consumption. The genes encoding the enzymes involved in anaerobic *p*-cresol degradation are widely spread over the genome and not arranged in an operon. A putative regulator has not been identified so far, but it was suggested that the regulation system for toluene might also be involved in *p*-cresol regulation.

The main aim of my work is the biochemical and physiologic characterization of the regulators of phenolic compound metabolism. This includes the analysis of transcription starts of the operons involved in the degradation processes as well as analyzing reporter gene constructs. Further plans include promoter shift experiments, e.g. substituting phenol and *p*-ethylphenol responsive promoters, and the construction of artificial biosensors to determine concentrations of phenolic compounds. The regulator proteins themselves will be purified and characterized. Further aims are the regulation mechanism of anaerobic *p*-cresol degradation, the functional characterization of a third XylR-like regulator adjacent to a "stress" operon and a comparison to the regulation of phenolic compound degradation of other anaerobic degrading bacteria (e.g. *Geobacter*).

Akinetes: resistant cells of Filamentous Cyanobacteria

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Akinetes are spore-like non-motile cells that differentiate from vegetative cells of filamentous cyanobacteria from members of the Nostocales (*N. punctiforme and A. variabilis*) and Stigonematales orders. They play a key role in the survival under changing environmental conditions like cold and starvation, and contribute to their perennial blooms. Various environmental factors were reported to trigger the differentiation of akinetes including light intensity and light quality, temperature and nutrient deficiency. Akinetes are larger and have a thicker cell wall than vegetative cells and they contain large amounts of reserve materials and DNA. During differentiation, akinetes of other strains accumulate glycogen and cyanophycin granules, a storage compound for N, which is composed from amino acids arginine and aspartate. In addition, metabolic and morphological changes take place in the maturation process.

In this study we investigated the morphological changes during the differentiation and germination processes of akinetes in *N. punctiforme* and *A. variabilis* in more detail. First, the best laboratory conditions to induce akinetes in both strains were investigated by testing dim light, low temperature and phosphorus starvation, revealing clear strain specificities. Morphological changes were investigated by light microscopy (LM) and transmission electron microscopy (TEM) of ultra thin sections. Light micrographs showed high amounts of big granules in akinetes induced by phosphorous starvation, but not by dim light in both strains. These structures may correspond to the cyanophycin granules observed in TEM pictures of akinetes. This was confirmed by measuring the cyanophycin concentration *in vitro* and staining of cyanophycin in the cell during the time curse of induction. In addition, during akinete differentiation, TEM pictures of *N. punctiforme* showed akinete like "unicellular spores" or sometimes in filaments, with different granules, rearrangement of thylakoid membrane and thicker cell wall. In the case of *A. variabilis*, also various granules were observed in the first stages of akinete differentiation as well as changes in cell wall thickness. In contrast to *N. punctiforme* akinetes were always like "unicellular spores" with a real coat envelope composed of different layers. Finally, the germination process in both strains was characterized by LM and TEM, showing first, changes in the akinete cell wall, followed by fast cell divisions inside of the akinete and subsequent expansion of the cells that resulted in rupture of the envelope. Finally small filaments consisting of vegetative cells emerged. In *A. variabilis* small filaments with terminal heterocyst were also observed. Currently we try to develop methods to identify genes involved in akinete formation and germination. With our profound knowledge of the morphological changes by our ultrastructural study we will be able to assign the roles of the identified genes to special developmental stages.

Nitrogen-starvation induced chlorosis in cyanobacteria: a program for maintenance of viability

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When non-diazotrophic cyanobacteria are deprived of combined nitrogen sources, cells degrade their photosynthetic pigments, a process that is termed chlorosis. These visible steps are part of an acclimation process to combined nitrogen deficiency, which is characterized by the sequential decay of metabolic activity and by a massive degradation of almost all cellular proteins. Several cyanobacterial species accumulate storage compounds during this metabolic transition. The unicellular cyanobacterial strain *Synechocystis* PCC 6803 accumulates, in addition to glycogen, poly-betahydroxybutyrate (PHB). In the final dormant state of chlorosis, most cells are packed with PHB and maintain traces of photosynthetic activity and de-novo protein synthesis, which enables rapid regeneration when nutrients become available. Thus, chlorosis is a model system to analyze dormancy-metabolism, recovery from dormancy and the biological role of storage polymers. Our current projects focus on the biological role of PHB formation and the recovery from chlorosis.