Closing Symposium

Ecology, Physiology and Molecular Biology of the *Roseobacter* group: Towards a Systems Biology Understanding of a Globally Important Group of Marine Bacteria

Program

Venue: Alter Landtag zu Oldenburg,
Theodor-Tantzen-Platz 8,
26122 Oldenburg

Organising committee:
Meinhard Simon, Katinka Hoppe

Contact: Meinhard Simon (m.simon@icbm.de),
Katinka Hoppe (katinka.hoppe@uol.de)

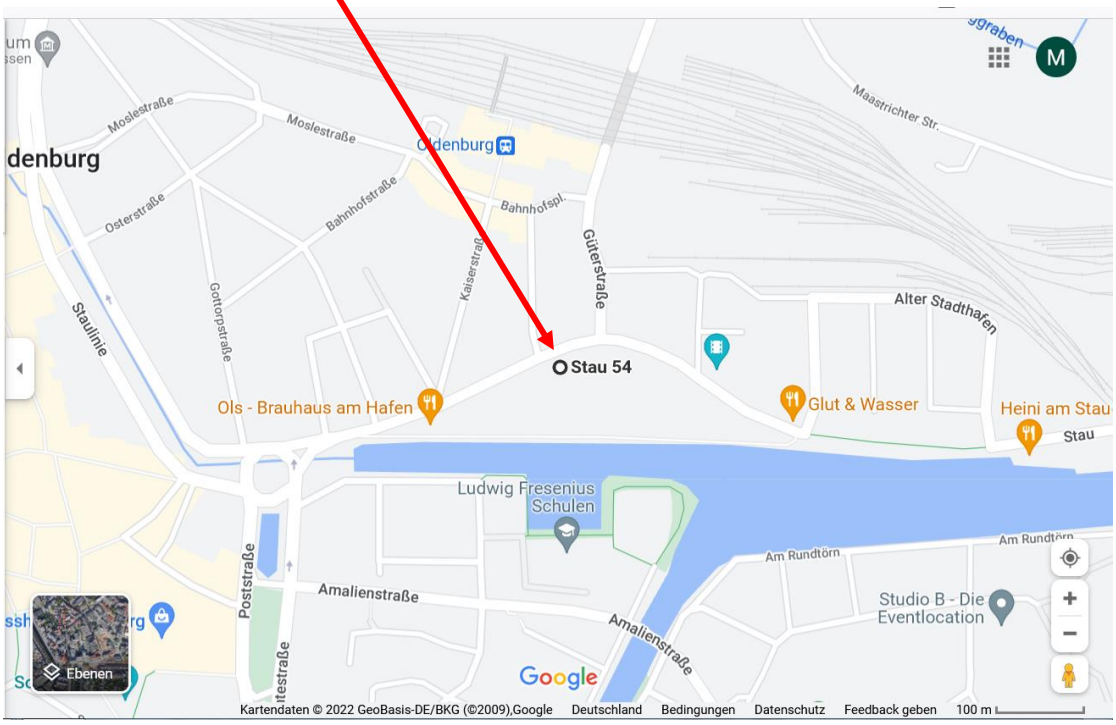
Program

4 September 2022, Sunday

Arrival
18:00 until around 21:00– informal get together in the restaurant Secco at the harbor in Oldenburg

SECCO am Hafen

Stau 54 - an der Hafenpromenade
26122 Oldenburg



5 September 2022, Monday

Time	*Subject*	*Speaker*
9:00-9:15	**Welcome and Introduction**	Meinhard Simon
9:15-9:45	A holistic perspective on marine microbial systems	Jed Fuhrman, USC Los Angeles
	Ecology and Evolution	
9:45-10:15	Ecological significance, biogeography and physiology of the *Roseobacter* group in pelagic systems	Meinhard Simon Felix Milke et al.
10:15-10:45	Distribution, metabolic capacities and phage-host interactions of the *Roseobacter* group in marine sediments	Marion Pohlner et al.
10:45-11:15	Coffee break	
11:15-11:45	Assessment and exploitation of the metabolic potential and molecular characterization of uncultivated members of the *Roseobacter* group	Rolf Daniel et al.
11:45-12:15	Extrachromosomal, extraordinary and essential: The biology of roseobacters from a plasmid point of view	Jörn Petersen, Silke Pradella et al
12:15-12:45	Adaptation of the *Roseobacter* group to the diatom phycosphere	Shady Amin, NYU Abu Dhabi
12:45-14:00	Lunch break	
14:00-14:30	Taxonomic and nomenclatural issues in *Alphaproteobacteria*, with special emphasis on the *Roseobacter* group	Markus Göker, Jan Meier-Kolthoff
14:30-15:00	Population structure and divergence in the *Roseobacter* group – implications for ecology and evolution	Heike Freese, Jörg Overmann
15:00-15:30	Studying transfer RNA transcripts to understand translational priorities	A. Murat Eren HIFMB Oldenburg
15:30-16:00	Coffee break	
16:00-16:30	Linking the exometabolome of pelagic organisms of the *Roseobacter* group to marine dissolved organic matter	Sarah Bercovici, Gerrit Wienhausen, et al.
	Genetics and Physiology	
16:30-17:00	Chemistry of secondary metabolite mediated interactions between bacteria of the *Roseobacter* group and other organisms	Stefan Schulz et al.
17:00-19:00	Poster session	
19:00	Symposium dinner	

6 September 2022 Tuesday

Time	*Subject*	*Speaker*
9:00-9:30	Adaptive mechanisms that provide competitive advantages to marine bacteria during microalgal blooms	Thomas Schweder, Univ of Greifswald
9:30-10:00	Function and ecological significance of secondary metabolites produced by *Roseobacter* spp. for interactive relationships	Thorsten Brinkhoff et al.
10:00-10:30	Sulfur metabolism in marine bacteria	Jeroen Dickschat et al.
10:30-11:00	Coffee break	
11:00-11:30	Evolution of resilience against heat-stress in a red-tide dinoflagellate	Irene Wagner-Döbler et al.
11:30-12:00	Regulatory networks for the adaptation of *Dinoroseobacter shibae* to changes in oxygen, iron and light	Dieter Jahn, Elisabeth Härtig et al.
12:00-12:30	Dual phototrophy in *Sphingomonas glacialis* AAP5 isolated from an alpine lake	Michal Koblizek, CAS, Trebon,
12:30-13:00	Using cultivation to discover new roseophage diversity in the coastal marine environment	Cristina Moraru et al.
13:00-14:00	Lunch break	
	Systems Biology	
14:00-14:30	Metabolic capacities and adaptability of *Phaeobacter inhibens* DSM 17395	Ralf Rabus et al.
14:30-15:00	Talking with molecules: Marine bacteria and microalgae	Mo Seyedsayamdost, Princeton University
15:00-15:30	Coffee break	
15:30-16:00	Metabolic characterization of *Prorocentrum cordatum*	Karsten Hiller et al.
16:00-16:30	The phyco-microbiome of *Prorocentrum cordatum* – friend or foe?	Irene Wagner-Döbler et al.
16:30-17:00	Influence of *Phaeobacter* spp. on marine algal health and microbiome assembly	Suhelen Egan, UNSW, Sydney
17:00-17:30	Modelling of physiological bioenergetics and global biogeography of the *Roseobacter* group	Bernd Blasius et al.
17:30-18:00	Temporal and spatial signaling in phytoplankton / bacteria interactions	Georg Pohnert, University of Jena
18:00-18:30	Closing remarks	

List of posters – order according to project number

A1

Tran Quoc Den, Thomas R. Neu, Sabiha Sultana, Helge-A. Giebel, Meinhard Simon, Sara Billerbeck:

Distinct glycoconjugate cell surface structures make the pelagic diatom *Thalassiosira rotula* an attractive habitat for bacteria

Leon Dlugosch, Anja Poehlein, Bernd Wemheuer, Birgit Pfeiffer, Thomas H. Badewien, Rolf Daniel, Meinhard Simon:

Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome.

Felix Milke, Irene Wagner-Döbler, Gerrit Wienhausen, Meinhard Simon:

Selection, drift and community interactions shape microbial biogeographic patterns in the Pacific Ocean.

A2

Dennis Alexander Tebbe, Charlotte Gruender, Martin Könneke, Bert Engelen, Hendrik Schäfer:

DMS from DMSO reduction fuels methanogenesis in salt marsh sediments

Benedikt Heyerhoff, Bert Engelen, Carina Bunse

Auxiliary metabolic gene functions in pelagic and benthic viruses of the Baltic Sea

A5

Lukas Birmes, Henner Brinkmann, Heike Freese, Jörn Petersen:

The astonishing wealth of RepABC-type plasmids in *Rhodobacterales*

Pia Marter, Sixing Huang, Heike M. Freese, Henner Brinkmann Jörn Petersen:

Metagenomic insights into the marine mat-forming cyanosphere – *Coleofasciculus* and associated heterotrophic bacteria.

A6

Jan Meier-Kolthoff and Markus Göker

Overall research highlights from project A6: Phylogenomics & functional genomics of the *Roseobacter* group

A8

Sabiha Sultana, Stefan Bruns, Heinz Wilkes, Meinhard Simon, Gerrit Wienhausen:

Vitamin B_{12} is not shared by all marine prototrophic bacteria with their environment

B2

Martine Berger, Kathrin Schäfer, Jan Tebben, Jan Meier-Kolthoff, Tilmann Harder, Markus Göker, Thorsten Brinkhoff:

Bacterial siderophore biosynthesis cluster traveling across genomes and environments

Sujatha Srinivas, Dmytro Spriahailo, Anja Poehlein, Rolf Daniel, Katharina Hoff, Tilmann Harder, Jan Tebben, Uwe John, Thorsten Brinkhoff

Response of a marine diatom to a bacterial signalling molecule

B7

Anuj Chhalodia and Jeroen S. Dickschat:
Sulfur metabolism in marine bacteria

C1

A. Weiten et al.
Nanomolar responsiveness of marine *Phaeobacter inhibens* DSM 17395 toward carbohydrates and amino acids.

C3

tba

C5
J. Kalvelage et al.
The enigmatic nucleus of the marine dinoflagellate *Prorocentrum cordatum*

C7

Leonhard Lücken and Bernd Blasius
Bioenergetics modelling of bacterial growth on a mixture of organic resources

Others

Jürgen Tomasch:

Clustered gene orientation bias on *Gemmatimonadota* chromosomes

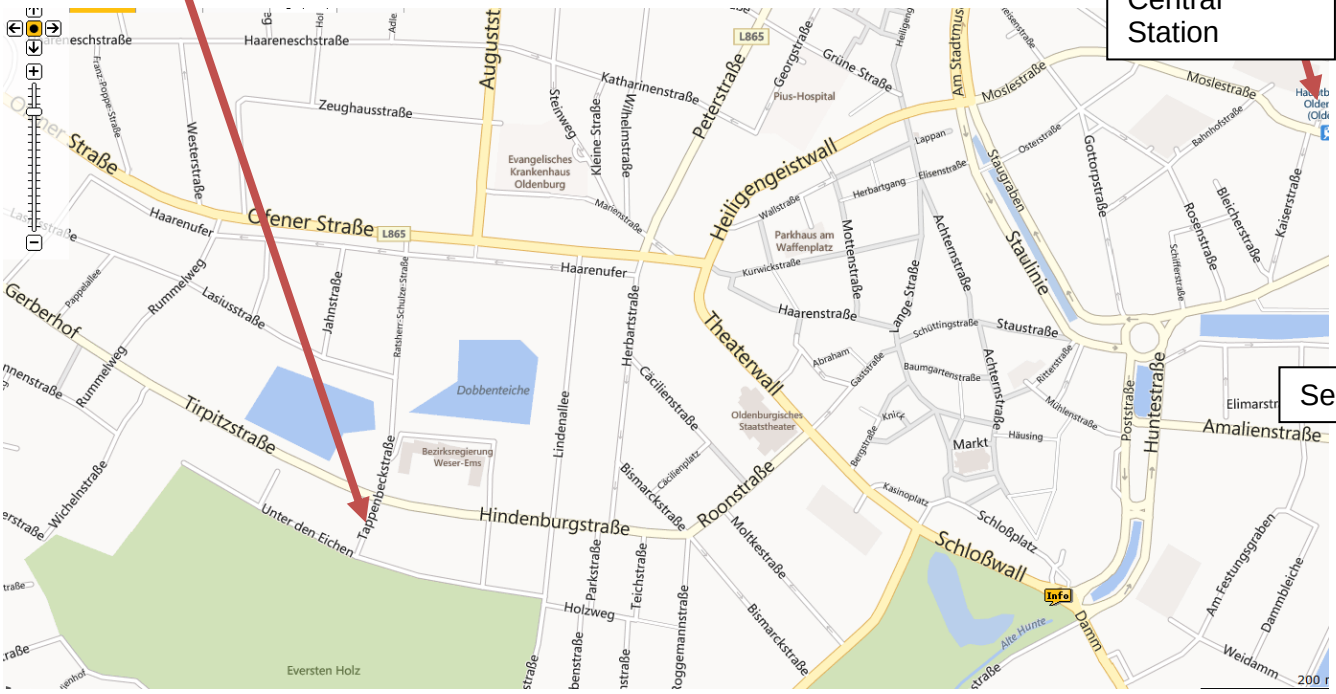
Location of the venue:

Alter Landtag (Old Parliament),

Theodor-Tantzen-Platz 8 (Tirpitzstraße Ecke Tappenbeckstraße)

26122 Oldenburg

Hauptbahnhof
Central
Station



Secco

Abstracts Guest Speakers

A holistic perspective on marine microbial systems

Jed Fuhrman, University of Southern California, Los Angeles, USA

We all recognize that microbial systems involve complex interactions among prokaryotes, eukaryotes, and viruses, though most studies rely on measurements and analyses that tend to focus on particular subgroups of interest, making it difficult to see the entire picture. This is a natural consequence of specialization among researchers as well as historical limitations on what could practically be measured. In our lab we have been using 3-domain primers backed with mock communities to make accurate quantitative analyses of the entire cellular community in a single PCR reaction, and metagenomes to assess viruses. The work includes the multi-decade SPOT time series off California where we have found remarkable stability on free-living prokaryotic and viral communities, intermediate stability in attached prokaryotes and phytoplankton, and the least stability in the overall protist community. Despite stability at the species level, viruses showed constantly changing strains, suggesting ongoing defenses and counter-defenses between viruses and hosts. We also noted the strongest statistical interactions among microbial types when examined at the strain level, suggesting interactions occur at fine phylogenetic resolution. Global ocean transects with unfractionated samples and 3-domain primer analyses have allowed us to show the changing ratios of prokaryotes to eukaryotes, and in fact the ratios of any taxon to any other taxon all on the same scale, something usually not possible with size fractionated samples or multiple primer analyses that have been common in previous global studies.

Adaptation of the *Roseobacter* group to the phytoplankton phycosphere

Shady Amin, New York University at Abu Dhabi

Interactions between phytoplankton and bacteria arguably represent the most important inter-species association in aquatic environments. These relationships influence fundamental processes that include nutrient provision and regeneration, primary production, harmful blooms and biogeochemical cycling. Although typically studied over large spatial and temporal scales, this relationship is governed by microscale interactions that take place within the region immediately surrounding phytoplankton cells known as the phycosphere. The phycosphere is enriched in phytoplankton-derived organic molecules that help attract, select, and nurture microbial communities that benefit phytoplankton cells. Among many groups of bacteria that colonize the phycosphere, the Roseobacter group is by far the best studied and the most adapted to this environment. In my talk, I will discuss examples of the molecular mechanisms that enable this group of bacteria to interact with their eukaryotic phytoplankton hosts. These mechanisms vary from rapid transcriptional and metabolic responses to algal exudates, some of which are toxic to other bacteria, the ability to colonize the phycosphere using quorum sensing and attachment and production of hormones that facilitate associations with phytoplankton. A better understanding of how these bacteria influence their algal hosts has wide implications for marine biogeochemistry and the carbon cycle that I hope to convey during my talk.

Studying transfer RNA transcripts to understand translational priorities

A. Murat Eren (Meren), Helmholtz Center for Functional Marine Biodiversity, Oldenburg

While all life scientists know about the canonical role of transfer RNAs (tRNAs) in protein synthesis, the past decade witnessed the emergence of groundbreaking studies that reveal additional roles of tRNAs in regulating translation. The fine tuning of translational processes through tRNAs is common to all three domains of life and allows living cells to influence gene expression beyond transcription, or yield proteins that are not quite encoded by the genome through mistranslation. This talk will offer a general overview of the role of tRNAs and our recent efforts to benefit from their unique properties to study how marine microbes respond to their changing environments.

Adaptive mechanisms that provide competitive advantages to marine bacteria during microalgal blooms

Thomas Schweder, University of Greifswald

Marine bacteria play an essential role in global carbon cycling. This is particularly evident during annually recurring massive algal blooms at upwelling zones and continental shelves in temperate latitudes, which perish as a result of enormous heterotrophic bacterial activities. In response to diatom-dominated phytoplankton blooms, marine surface bacterioplankton composition changes characteristically over time, with a number of dedicated genera of the class *Flavobacteriia* within the phylum *Bacteroidota* and distinct *Gammaproteobacteria* successively degrading complex algal polysaccharides and replacing each other in the process. To uncover what drives this bacterial succession we have to understand how bacteria compete for these abundant energy substrates. We need to determine specific physiological activities of the bacterial microbiome during microalgal blooms *in situ* and to unravel crucial molecular biological processes of key microbes *in vitro*. In this talk, specific bacterial polysaccharide utilization mechanisms such as sophisticated protein machineries will be presented and putative molecular adaptation processes which could provide competitive advantages to these marine bacteria during microalgal blooms will be discussed.

Dual phototrophy in *Sphingomonas glacialis* AAP5 isolated from an alpine lake

Michal Koblížek[1], Karel Kopejtka[1], David Kaftan[1], Alastair T. Gardiner[1], David Bína[2], Jürgen Tomasch[1]

[1]Laboratory of Anoxygenic Phototrophs, Institute of Microbiology of the Czech Acad Sci, 379 81 Třeboň, Czechia

[2]Institute of Plant Molecular Biology, Biology Centre, Czech Acad Sci, 370 05 České Budějovice, Czechia

Bacterium *Sphingomonas glacialis* AAP5 isolated from the alpine lake Gossenköllesee contains genes for anoxygenic phototrophy as well as proton-pumping xanthorhodopsin. *Sphingomonas* bacteria with the potential for dual phototrophy were identified in metagenomes collected from lake Gossenköllesee during winter and spring season, which indicates that these organisms are active at low temperatures. Therefore we investigated the influence of light and temperature on AAP5 gene expression. Xanthorhodopsin was expressed when illuminated at temperatures below 16°C. In contrast genes for anoxygenic phototrophy were expressed between 4 and 22°C in the dark. Thus, cells grown at lower temperature under natural light-dark cycle produced both xanthorophopsin and bacteriochlorophyll-containing reaction centers and used them simultaneously for energy generation. Xanthorhodopsin contains carotenoid nostoxanthin serving as an auxiliary antenna, which extends its absorption properties in a blue part of the spectrum. Upon illumination, xanthorhodopsin-containing cells reduced respiration, increased ATP synthesis and showed enhanced growth. This suggests, that dual phototrophy may represent a metabolic advantage in alpine lakes where photoheterotrophic organisms face limited organic substrates, low temperature, and extreme changes in irradiance.

Talking with Molecules: Marine Bacteria and Microalgae

Mo Seyedsayamdost, Princeton University, Princeton, New Jersey USA,

Although microbes are routinely grown in monocultures in the laboratory, they are almost never encountered as single species in the wild. Our ability to detect and identify new microorganisms has advanced significantly in recent years, but our understanding of the mechanisms that mediate microbial interactions still lags behind. What makes this task more challenging is that microbial alliances can be dynamic, consisting of multiple phases. The transitions between phases, and the interactions in general, are often mediated by a chemical language consisting of small molecules, also referred to as secondary metabolites or natural products. In this microbial lexicon, the molecules are like words and through their effects on recipient cells they convey meaning. In this talk, I will present the lessons we have learned from investigating naturally occurring symbioses between bacteria belonging to the roseobacter clade and microscopic algae. The lessons learned and the methods that have been developed provide a playbook for examining more complex bacterial-eukaryote interactions in diverse ecological niches.

Influence of *Phaeobacter* spp. on marine algal health and microbiome assembly

Suhelen Egan, University of New South Wales, Sydney, Australia

Members of the Roseobacter group are commonly found in association with marine algal hosts where they play important roles that can be both beneficial and at times detrimental to the host. In this seminar I will firstly present some of our recent work aiming to understand bacterial causes of bleaching disease in the red seaweed *Delisea pulchra* and the discovery of new *Phaeobacter* isolates with seaweed probiotic activity. Secondly, I will discuss studies showing that members of the Roseobacter group are effective colonisers of different algal surfaces and can influence subsequent microbiome development of the host. These findings not only demonstrate the ecological importance of these marine bacteria but also pave the way for effective microbiome manipulation strategies for conservation and/or biotechnology applications.

Temporal and spatial signaling in phytoplankton / bacteria interactions

Georg Pohnert, Friedrich Schiller University of Jena, Germany

The annual patterns of plankton succession in the ocean determine ecological and biogeochemical cycles. The temporal fluctuating interplay between photosynthetic eukaryotes and associated microbiota balances the aquatic ecosystems' composition. Besides nutrients and abiotic factors, chemical signaling mediates the interactions between phytoplankton and its associated microbiome. In this talk chemical mediators are introduced that drive the species succession and community composition across time and space in processes that are highly dynamic. We introduce chemical analysis, laboratory co-culturing, mesocosm- and field studies to unravel the role of small molecules in the regulation of the plankton microbiome.

Oral presentations of CRC Projects

A1

Ecological significance, biogeography and physiology of the *Roseobacter* group in pelagic systems

Felix Milke, Yanting Liu, Leon Dlugosch, Sara Billerbeck, Tran Quoc Den, Thorsten Brinkhoff, Meinhard Simon
ICBM, University of Oldenburg

The major aims of the third funding phase of this project are 1) a global assessment of the biodiversity and functional biogeography of pelagic *Roseobacter* clusters as part of the prokaryotic pelagic communities in the Atlantic and Pacific Ocean, and 2) interactions of roseobacters and other prokaryotes with the diatom *Thalassiosira rotula*.
In this presentation we will focus on the global patterns of the functional and taxonomic patterns of microbial communities with a special emphasis on the *Roseobacter* group. We characterized the composition of prokaryotic and eukaryotic microbial communities in the epipelagic and upper mesopelagic Atlantic and Pacific Ocean between subantartic and temperate to subarctic regions. Homogenous selection, drift and community interactions, derived from co-occurrence patterns, were identified as the most important ecological mechanisms shaping biogeographic patterns. Both oceans share many features regarding biogeographic patterns and community composition, but exhibit also ocean-specific features, in particular in the northern and southernmost regions and in the upper mesopelagic.
A global biogeographic assessment of pelagic roseobacters based on more than 550 quality-controlled metagenome assembled genomes (MAG) and single cell amplified genomes (SAG) from all available metagenomics data sets of the global oceans revealed distinct distribution patterns for several sublineages including the RCA cluster, HIMB11, NAC11-7, Sulfitobacter and four so far unknown new lineages. All of them exhibit features of genomic streamlining with a smaller genome size than most other roseobacters known from isolates and a low GC-content. Surprisingly, almost all of these lineages included members encoding the proteorhodopsin gene, a trait for energy acquisition known so far only from the NAC11-7 cluster and two MAG of the RCA cluster. Our analysis provides a detailed insight into the functional features and how they reflect the global biogeography of the pelagic roseobacter lineages.

A2

Distribution, metabolic capacities and phage-host interactions of the *Roseobacter* group in marine sediments

Marion Pohlner, Benedikt Heyerhoff, Dennis Tebbe, Saranya Kanukollu, Bert Engelen
ICBM, University of Oldenburg

Members of the *Roseobacter* group represent a numerically significant part not only of pelagic, but also of benthic microbial communities. In our project, we want to understand the biogeography and metabolic functions of roseobacters in marine sediments as well as their interaction with phages. Therefore, the microbial communities of sediments from different oceanic regions were analyzed regarding their diversity by cultivation-based and molecular approaches. In the North Sea, distinct communities of free-living and particle-associated roseobacters were found, while the community composition of *Roseobacter*-affiliated bacteria at the sediment surface was highly similar to the one on old, matured particles. In sediments of Antarctica and the Pacific Ocean, roseobacters accounted for 1 - 2% of all bacteria and the genera *Sedimentitalea*, *Boseongicola* and *Sulfitobacter* were typical benthic representatives among a large proportion of uncultured members. Linking the distribution of the *Roseobacter* group to environmental factors in a dataset of global sediments demonstrated that one-third of the *Rhodobacteraceae*-OTUs significantly correlated to the prevailing redox regime, suggesting an adaption to anoxic conditions. As isolation of several new strains was successful using media containing organosulfur compounds, the metabolic capabilities of the *Roseobacter* group were investigated with a special emphasis on their contribution to the sulfur cycle. Investigation of the land - sea transition zone in a salt marsh at the North Sea coast indicated a seasonal and zonal succession of bacterial communities. Furthermore, it could be shown that DMS from DMSO reduction fuels methanogenesis in those sediments. As benthic roseobacters are influenced by phages, also their role, abundance, genetic composition and lifestyle are investigated. As a first step the diversity, distribution and composition of marine viruses were analyzed in publically available metagenomes from the Baltic Sea with a special focus on auxiliary metabolic genes originating from virus infections. It could be found that the predominant viral lifestyle is lytic, but lysogeny was more prevalent in sediments than in pelagic samples. Overall, we gained deeper insights into the biogeography of the *Roseobacter* group and their role in marine sediments, but the involvement of this group in the cycling of organosulfur compounds and their interaction with phages needs to be investigated in further detail.

A5

Extrachromosomal, extraordinary and essential – The biology of roseobacters from a plasmid point of view

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

More than fifteen years of research on extrachromosomal replicons (ECRs) reflect the pivotal role of plasmids for the evolution, biology and ecology of *Roseobacteraceae*. The discovery of a dozen ECRs in *Marinovum algicola* ["*Ocean´s twelve"*] provided general insights into the multipartite genome organization of *Alphaproteobacteria*. Stably evolving chromids are diagnostic markers of ancient evolutionary adaptations, which could be exemplified by biofilm, flagellar and even photosynthesis chromids of the genus *Roseobacter* ["*Think pink"*]. Mobile plasmids mediate rapid adaptions to a changing environment ["*Plasmid transfer in the Ocean"*]. Novel promiscuous plasmid systems (RepL, RepC_soli) illustrated the frequent exchange of chromate- and chloramphenicol-resistance genes in the ocean. They also documented the connectivity of the environment, livestock breeding and human pathogens in the light of the 'One Health' concept.

Holistic insights into the functional role of ECRs were either obtained from specific knock-outs ["*Plasmid curing and the loss of grip"*] or by plasmid conjugation ["*Fatal affairs"*]. The respective mutants of the model organisms *Phaeobacter inhibens* DSM 17395 and *Dinoroseobacter shibae* DFL 12 were the focal point of systems biology in the CRC and lead to astonishing results. *Phaeobacter* spends up to a third of its total energy budget for the biosynthesis and detoxification of the chromid-encoded antibiotic tropodithietic acid ["*The limits to growth"*], but this investment ensures a unique access to nutrients in marine biofilms. A 102-kb plasmid of *D. shibae* has tremendous effects on the expression of chromosomal genes ["*The sixth element"*] and co-cultivation experiments with the dinoflagellate *Prorocentrum minimum* allowed to identify a 191-kb killer plasmid ["*Jekyll and Hyde Interaction*"].

RepABC-type replicon are the most abundant and diverse group of low-copy number plasmids in *Rhodobacterales*. The identification of more than a dozen compatibility groups was the basis for the current development of cloning vectors for future biotechnological applications in roseobacters and rhizobia.

A6

Taxonomic and nomenclatural issues in *Alphaproteobacteria*, with special emphasis on the *Roseobacter* group

Markus Göker, Jan Meier-Kolthoff
Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig

The "*Roseobacter* group", a more or less well defined ecologically important assemblage of Gram-negative bacteria, had been taxonomically assigned to the family *Rhodobacteraceae*, the order *Rhodobacterales*, the class *Alphaproteobacteria*., and the phylum "*Proteobacteria*". The entire class was affected by taxonomic and nomenclatural problems. As revealed by the analysis of draft genome sequences of a collection of genomes of more than 1000 *Alphaproteobacteria* and outgroup type strains, the majority of taxa were monophyletic but several orders, families and genera, were in need of revision. This included taxa recognized as problematic long ago but also quite recently proposed ones. Several names of taxa within *Alphaproteobacteria*, while validly published under the International Code of Nomenclature of Prokaryotes (ICNP), contravene the regulations of this Code and are therefore illegitimate. This includes *Rhizobiales* and *Rhodobacteraceae*. The recent proposal of a family *Roseobacteraceae* did not consider that the phylogenetic placement of the family makes *Rhodobacteraceae* paraphyletic, which calls for a solution in agreement with the ICNP. The name *Alphaproteobacteria* itself had been illegitimate until a recent decision of the International Committee on Systematics of Prokaryotes (ICSP) made Rule 8 non-retroactive regarding names of classes. However, further measures are needed because the name *Alphaproteobacteria* remained illegitimate anyway. The proposal to include phylum names into the ICNP is also of relevance in this context, as is the taxonomic dissection of "*Proteobacteria*".

A7

Population structure and divergence in the *Roseobacter* group – implications for ecology and evolution

Heike M. Freese, Jörg Overmann
Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig

The Roseobacter group is one of the major groups in the ocean and its broad functional capacity is sustained by a high diversity of bacterial species. Bacteria associated with particles or eukaryotic organisms show a higher diversity, which may be an adaptation to the microscale heterogeneity of their environment. This raises questions about the extent of genomic diversity of species with this substantially understudied lifestyle, their evolution and potential adaptation to ecological niches. Therefore, we elucidated the population structure and evolution of the surface-associated *Phaeobacter* based on the closed genomes of 35 isolates. They share an exceptional large core genome but their chromosomes were continuously though slightly expanding over evolutionary time. The strains form clearly delineated phylogenomic clades independent from their geographic distribution. Clade-specific differences indicate adaptive advantages to different ecological niches and divergence could be related to a limited number of acquired genes. Furthermore, all strains harbor three chromids, which were already present in the last common ancestor and stably co-evolved with the chromosome during speciation and probably even contribute to the divergence of the clades. In contrast, plasmids of *Phaeobacter* mediated more recent and sporadic habitat-specificity for instance via chloramphenicol resistance contributing to the spread of antibiotic resistance in the ocean. Our study reveals a comprehensive picture about the evolution of a surface-associated roseobacter, which seem to differ notably from other bacteria. To investigate whether these evolutionary trajectories differ among members of the Roseobacter group that have different lifestyles, we investigated the generalist *Sulfitobacter*, which revealed some remarkable traits. The highly diverse genus did not contain genus specific chromids but the unique event of a chromid acquisition triggered the divergence of a subgroup. Furthermore, ribosomal genes were subject to elevated recombination, which implies that a 16S rRNA gene classification may not always be sufficient for speciation and microdiversity research.

A8

Linking the exometabolome of pelagic organisms of the *Roseobacter* group to marine dissolved organic matter

Sara Bercovici, Gerrit Wienhausen, Sabiha Sultana, Thorsten Dittmar, Meinhard Simon, Jutta Niggemann
ICBM, University of Oldenburg

During the last funding phase, the aims of this project are twofold. One aim is the evaluation and multivariate statistical interpretation of the complex data sets collected during the previous funding phases, both from laboratory experiments and from oceanic cruises. Jutta, please add.
The other aim is to study specifically interactions of *Roseobacter* strains with other pelagic bacteria and diatoms. Here we focus on vitamin B_{12}, a key growth factor for marine microbial communities but produced only by a minority of prokaryotes including the *Roseobacter* group. We provide experimental evidence that only about half of the B_{12}-producers of *Roseobacters* and *Gammaproteobacteria* release this growth factor and share it with other microorganisms whereas the others retain it intracellularly. We further show that two bacterial strains, a *Colwellia* sp. and a *Roseovarius* sp., each capable of producing only one of the two building blocks of B_{12}, together are able to synthesize B_{12} and provide a diatom for sustained growth with this growth factor. Searches in the Atlantic Ocean metagenome data set reveal that these B_{12}-auxotrophic genomic features are well represented in distinct biogeographic regions. We further studied prototrophic and auxotrophic features of biotin (vitamin B_7) in *Roseobacters* and other prokaryotes which are auxotrophic for this vitamin. Quite a few of these bacteria are able to salvage B_7 auxotrophy when they genomically only encode the gene for the last biosynthetic step from desthiobiotin to biotin. This genomic feature is also prevalent in the metagenomes of the more productive regions of the Atlantic Ocean.

B2

Function and ecological significance of secondary metabolites produced by *Roseobacter* spp. for interactive relationships

Thorsten Brinkhoff, Marco Dogs, Paul Beyersmann, Martine Berger, Laura Wolter, Janina Leinberger, Sujatha Srinivas
ICBM, University of Oldenburg

Numerous representatives of the *Roseobacter* group, i.e. the marine *Rhodobacteraceae*, are prominent colonizers of surfaces, and many of these organisms are associated and interact with other bacteria, algae and invertebrates. This is partially facilitated by production of a wealth of secondary metabolites, which were frequently detected during the last years. This includes a variety of acyl-homoserine lactones (AHLs), often observed in associated Roseobacters. AHLs mediate quorum sensing (QS) and allow the organisms to interact with their environment in a density dependent manner. Several Roseobacters can produce antibiotics like tropodithietic acid (TDA), detected exclusively in members of the genera *Phaeobacter*, *Ruegeria*, *Epibacterium* and *Pseudovibrio*. Within the *Roseobacter* group, TDA is probably the most intensively studied secondary metabolite. TDA is a broad spectrum antibiotic that additionally acts as signalling molecule at subinhibitory concentrations. Whole-transcriptome analyses and phenotypic screenings of the model organism *Phaeobacter inhibens* showed that TDA causes the same regulatory effects in QS as the common signalling molecule AHL, but at concentrations 100-fold lower than the minimal inhibitory concentration against bacteria. Thus, low concentrations of antibiotics can obviously have a strong influence on the global gene expression of the bacterium that produces it, and drastically change the metabolism and behaviour of the bacterium. Furthermore, chemotactic effects of subinhibitory concentrations of TDA on different *Rhodobacteraceae* were observed, contextualized with the influence on gene regulation in *P. inhibens*. TDA attracted TDA-producing strains, but repelled a non-producing strain. Addition of 10 µM TDA upregulated multiple genes for anabolic processes and mutual interaction in *P. inhibens*. The diverse chemotactic responses demonstrate different strategies of surface colonization and chemical crosstalk in *Rhodobacteraceae*, with implications for biofilm dynamics and host association. The latter was confirmed, showing growth stimulating effects of the diatom *Thalassiosira rotula* by addition of subinhibitory concentrations of TDA to the culture. This observation was accompanied by strong transcriptomic changes of the diatom, indicating a sophisticated interaction between TDA producers and their hosts.

B4

Evolution of resilience against heat-stress in a red-tide dinoflagellate

Irene Wagner-Döbler Johannes Mansky, Selene Sanchez-Garcia, Hui Wang & *Prorocentrum cordatum* genome sequencing consortium**.**
Institute for Microbiology; University of Braunschweig, Braunschweig

We present the first sequenced genome of a bloom-forming “red tide” dinoflagellate, *Prorocentrum cordatum*, and the transcriptome, proteome, and metabolome data from axenic cell cultures to elucidate their molecular responses to heat stress. Comparing against available genomes of free-living and symbiotic dinoflagellates, we identified genomic hallmarks of a bloom-forming species. We adopted an integrated multi-omics approach to assess differential expression of genes and proteins, and recovery of metabolites relative to heat-stress response. These results lead to fundamental insights into ecology and evolution of dinoflagellates. This is the first integrated analysis of heat-stress response in a dinoflagellate in the absence of a microbial community or a host organism, i.e. in exclusively phototrophic conditions. As such, our observations are bona fide responses from the dinoflagellate.

B5

Regulatory networks for the adaptation of *Dinoroseobacter shibae* to changes in oxygen, iron and light

Elisabeth Härtig and Dieter Jahn
Institute for Microbiology; University of Braunschweig, Braunschweig

The marine bacterium *Dinoroseobacter shibae* DFL12T is a member of the Roseobacter group, which are highly abundant in the marine ecosystem and possess a large metabolic diversity. *D. shibae* utilizes aerobic respiration, anaerobic denitrification as well as aerobic anoxygenic photosynthesis for energy generation. The photosynthetic pathway involves more than 45 genes which are organized in a 47 kb gene cluster and the expression is regulated in a light-dependent manner. A genome-wide screen of the transposon library of *D. shibae* for loss of pigmentation and specific bacteriochlorophyll absorbance, identified the gene encoding the blue light-dependent LOV-protein Dshi_1135 and the *clpX* gene encoding the ATPase subunit of the ClpXP protease. The mutant phenotype of the Dshi_1135::Tn mutant strain could be complemented by expression of a Dshi_1135-Strep fusion protein *in trans.* Purified Dshi_1135 binds FMN and is able to perform a blue-light induced photocycle typical for LOV domain proteins. Transcriptome analyses revealed an essential role of Dshi_1135 as well as ClpX for expression of the photosynthetic gene cluster. An *in vivo* co-affinity purification experiment identified the repressor protein PpsR as interaction partner of Dshi_1135. This interaction between PpsR and the Dshi_1135 Protein was verified using the bacterial adenylate cyclase two hybrid assay. Moreover, we could demonstrate that cobalamin is essential for interaction of PpaA with PpsR and thus for antirepressor function. To prove the postulated antirepressor function of Dshi_1135 we established an *in vivo* test system in*E. coli*. Here, the expression of a *bchF-lacZ* reportergene fusion was used to monitor repressor function of PpsR and light-dependent antirepressor function of Dshi_1135. These results contribute to the functional understanding of the regulation of light-dependent expression of the photosynthetic gene cluster in *D. shibae* and describe the role of the newly identified blue light-dependent regulatory protein Dshi_1135.

B6

Using cultivations to discover new roseophage diversity in the coastal marine environment

Falk Zucker, Vera Bischofff, Cristina Moraru*
ICBM, University of Oldenburg

The phage diversity in the marine environment has been estimated to encompass hundreds of thousands of species. Most often, metagenomic techniques, in which viral genomes are assembled from a pool of environmental phages, are used to access this diversity, due to their high throughput abilities. Cultivation methods, on the other hand side, are much more laborious and offer a comparatively low throughput. However, their advantages come, for example, from the ability to directly link phages with their hosts, even down to the strain level, to conect genomic content with infection characteristics and phage morphology, and to offer model systems for the study of phage-host interactions. Within the Roseobacter SFB, “Bacteriophages of Roseobacter Group” project, we have focused on the large-scale isolation of roseobacter infecting phages from the North Sea. This work led to the characterization of more than one hundred phages. We have found both dsDNA and ssDNA phages. While some phages were related to previously cultivated phages, many were completely new. Using genome-based classification tools recently developed in our group, for example, VIRIDIC and VirClust, we delineated new dsDNA and ssDNA phage families and subfamilies. Overall, this work shows how phage cultivations can still be used to shed light on new and diverse roseophage branches, and significantly extend their known diversity space.

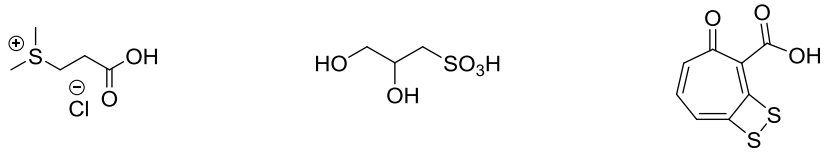
B7

Sulfur Metabolism in Marine Bacteria

Jeroen S. Dickschat,[1] Anwei Hou, Immo Burkhardt, Lukas Lauterbach, Patrick Rabe, Jan Rinkel, Anuj K. Chhalodia, Nelson L. Brock, Christian Citron, Ramona Riclea, Ersin Celik, Dorota Konvalinkova, Julia Gleitzmann, Hilke Bruns, Alexander Nikolay, Claudia Zell and Markus Menke,
in collaboration with Stefan Schulz, Irene Wagner-Döbler, Meinhard Simon, Thorsten Brinkhoff, Jörn Petersen, Robin Teufel, Erhard Bremer, Lone Gram and coworkers

[1] Kekulé-Institute for Organic Chemistry and Biochemistry, University of Bonn

Sulfur is one of the most important elements of life. In primary metabolism it occurs in amino acids (cysteine and methionine), and its special properties are often relevant for enzyme catalysis, e. g. the high nucleophilicity of the thiol group is often used through direct involvement of Cys in enzyme transformations. Also many enzyme cofactors contain sulfur, including coenzyme A, *S*-adenosylmethionine, biotin and lipoic acid. Major sulfur metabolites in the marine environment include dimethylsulfoniopropionate (DMSP, **1**)[1-3] and 2,3-dihydroxypropane-1-sulfonate (DHPS, **2**).[4] Their metabolism by marine bacteria and algae, including the involvement of these compounds in the biosynthesis of secondary metabolites such as the antibiotic tropodithietic acid (**3**)[5-8] from *Phaeobacter inhibens* will be discussed.



1 **2** **3**

Figure 1. Sulfur metabolites in marine bacteria and algae.

References

[1] I. Burkhardt, L. Lauterbach, N. L. Brock, J. S. Dickschat, *Org. Biomol. Chem.* **2017**, *15*, 4432.
[2] A. K. Chhalodia, J. S. Dickschat, *Beilstein J. Org. Chem.* **2021**, *17*, 569-580.
[3] J. S. Dickschat, C. Zell, N. L. Brock, *ChemBioChem* **2010**, *11*, 417.
[4] E. Celik, M. Maczka, N. Bergen, T. Brinkhoff, S. Schulz, J. S. Dickschat, *Org. Biomol. Chem.* **2017**, *15*, 2919-2922.
[5] V. Thiel, T. Brinkhoff, J. S. Dickschat, S. Wickel, J. Grunenberg, I. Wagner-Döbler, M. Simon, S. Schulz, *Org. Biomol. Chem.* **2010**, *8*, 234-246.
[6] M. Berger, N. L. Brock, H. Liesegang, M. Docks, I. Preuth, M. Simon, J. S. Dickschat, T. Brinkhoff, *Appl. Environ. Microbiol.* **2012**, *78*, 3539-3551.
[7] N. L. Brock, A. Nikolay, J. S. Dickschat, *ChemComm* **2014**, *50*, 5487-5489.
[8] Y. Duan, M. Toplak, A. Hou, N. L. Brock, J. S. Dickschat, R. Teufel, *J. Am. Chem. Soc.* **2021**, *143*, 10413-10421.

C1

Metabolic capacities and adaptability of *Phaeobacter inhibens* DSM 17395

Ralf Rabus, Kristin Kalvelage, Arne Weiten, Lars Wöhlbrand, Daniel Wünsch
ICBM, University of Oldenburg

The overarching aim of this subproject during the CRC was to decipher the physiological-metabolic capacities and adaptability of the heterotrophic model roseobacter *Phaeobacter inhibens* DSM 17395, which may provide general leverages for habitat success. Essentially, we pursued three major lines of research centering around plasmids, nitrogen and catabolism, respectively, integrating growth physiology with differential proteomics and metabolomics. (i) Taking into account the ample presence of plasmids (chromids) in roseobacters, we studied plasmid-cured mutants of *P. inhibens*, revealing that carriage of the large 262 kb plasmid contributed approx. 50% to its energetic (dissimilatory) expenditure and that the different replicons are interconnected on a functional as well as regulatory level. (ii) *P. inhibens* displayed maximal growth rates at external N:P ratios far above Redfield and adapts its internal N:P stoichiometry to the external nutrient supply. Following up on the relevance of nitrogen as nutrient, we found that *P. inhibens* secures external ammonium by rapid buildup of intracellular nitrogen stocks, concurrently cross-regulating the internal C/N/S-cycles. (iii) Initial time-resolved studies with complex substrates revealed dynamic utilization patterns of amino acids. Subsequent detailed analysis of the degradation routes for amino acids and carbohydrates allowed construction of catabolic networks which are build from substrate-specifically regulated modules, are widespread among roseobacters and differ in their efficiencies of carbon assimilation. The nanomolar responsiveness of *P. inhibens* toward selected carbohydrates and amino acids has implications for the strains viability under organic carbon poor conditions as well as for the emergent recalcitrant concept of DOM. Taken together, *P. inhibens* possesses a wide range of metabolic, sensory and regulatory capacities to respond to and be resilient under fluctuating environmental conditions as encountered in its natural habitat.

C2

Chemistry of secondary metabolite mediated interactions between bacteria of the *Roseobacter* group and other organisms

Stefan Schulz, Hilke Bruns, Lisa Ziesche, Diana Koteska, Alexander Neumann, Jonas Holste
Institute for Organic Chemistry, University of Braunschweig

Quorum-sensing is a wide spread phenomenon initially associated with *N*-acylhomoserine lactones (HSL) and common within roseobacters. HSLs were believed to occur in species specific mixtures allowing specific bacterial signaling. A broad investigation within the Roseobacter group bacteria revealed this to be only partly true because several structural design limits have been observed. In addition, closely related N-acyl amino acid esters such as NAMEs have been found, especially in Roseovarius. Additionally, other compounds were detected which are not unique to reoseobacters, nevertheless might be important in their ecology and physiology. An overview on these compounds will be given.

C3

Metabolic characterization of *Prorocentrum cordatum*

Karsten Hiller, Kerstin Schmidt-Hohagen
Institute for Biochemistry, Biotechnology and Bioinfomatics, University of Braunschweig

tba

C5

The phyco-microbiome of *Prorocentrum cordatum* – friend or foe?

Irene Wagner-Döbler, Selene Sanchez-Garcia, Johannes Mansky, Hui Wang
Institute for Microbiology; University of Braunschweig, Braunschweig

The phyco-microbiome is essential for micro-algae growth and bloom development. To decipher the interactions between bacteria and algae in the red-tide dinoflagellate *P. cordatum* we analysed a defined dual species co-cultivation system consisting of the roseobacter model organism *D. shibae* and the axenic strain *P. cordatum* CCMP1329. Our data show that vitamin B12, for which the algae is auxotrophic, and vitamin B7, for which both are auxotrophic, determine the outcome of this co-culture, namely the mutualistic and pathogenic phases of growth, respectively. Dual RNAseq of both dinoflagellate and bacterium showed KEGG pathways upregulated under mixotrophic growth conditions in the dinoflagellate, a key for understanding bloom development.
We then studied the bacterial community of 4 xenic strains of *P. cordatum.* They harbored a strain-specific phyco-microbiome and despite having been sub-cultured for up to 30 years they shared a core-microbiome consisting of typical algae-associated taxa. At increased temperatures, both core and specific taxa showed changes in abundance. Growth of all four xenic strains was impaired in comparison to the axenic CCMP1329, and only two of them were able to grow at 30°C like the axenic strain. The data suggest that competition for essential nutrients, including but not limited to vitamins, may govern the interaction between the dinoflagellate and its microbiome, resulting in a thin line between mutualism and pathogenicity. The data also suggest that axenic strains, if available, provide a more reliable picture when determining growth rate and temperature range of a dinoflagellate.

C7

Modelling of physiological bioenergetics and global biogeography of the *Roseobacter* group

Bernd Blasius,
ICBM, University of Oldenburg

The presentation will introduce a recently developed model framework that captures the interaction between a complex mixture of DOM compounds and a diverse community of microbial consumers as bipartite networks of DOM release and microbial turnover. Extending classic consumer-resource systems, the model yields surprising rich dynamic structure, including parameter regimes with chaotic dynamics, suggesting that microbial communities in the deep sea are characterized by self-organized temporal fluctuations. Including evolutionary processes, the model predicts a strong diversification of externally supplied DOM that creates niches for invasion of new microbial consumers, yielding cascades of subsequent extinctions of others. This leads to complex co-evolutionary dynamics subject to persisting turnover, even in stable environments. Thereby, microbial communities self-organize into different modules, akin to trophic layers in food-webs, and the system evolves to a state of highly diluted and diverse DOM in which micro-heterotrophs are living at the edge of their fitness range. These model results provide a mechanistic understanding of how the huge recalcitrance and diversity of DOM might emerge from the complex interactions between microbial communities and organic molecules. Finally, I show how implementing the DOM-microbe interactions into a global ocean model allows to capture large-scale DOM patterns in the ocean and to model expected changes of the DOM inventory in future climate scenarios.

Z02

Assessment and exploitation of the metabolic potential and molecular characterization of uncultivated members of the *Roseobacter* group

Rolf Daniel
Genomics Laboratory, Institute for Microbiology and Genetics, Georg-August-Universität Göttingen,

This project analyses ecosystem function and role of Roseobacter communities and environmentally abundant members of this group in the North Sea, Atlantic Ocean, Southern Ocean, and Pacific as well as in samples from aggregate-associated microbial communities by (meta)genomic and (meta)transcriptomic approaches. Data with respect to composition and function of entire (DNA level) and active communities (RNA level) under changing environmental conditions including regional (biogeographic) as well as water depth-dependent aspects will be presented. This will include changes in species richness, abundance, and distribution of *Roseobacter* groups and key functions as well as biogeographic aspect. The response of entire and active bacterioplankton communities to DFAA turnover and other factors are assessed by combining structural and functional predictions and validated by metagenomic analysis. Also results on analysis of activity of environmentally abundant but by genomes or isolates under represented Roseobacter clusters are provided. In addition, the functional role and distribution of an encountered novel xanthorhodopsin-type in *Octadecabacter* strains are presented.

Posters

A1

Significance of gene variants for the functional biogeography of the near-surface Atlantic Ocean microbiome

Leon Dlugosch[1], Anja Poehlein[2], Bernd Wemheuer[2], Birgit Pfeiffer[2], Thomas H. Badewien[1], Rolf Daniel[2], Meinhard Simon[1,3] *

[1] ICBM, University of Oldenburg,
[2] Göttingen Genomics Laboratory, Institute of Microbiology and Genetics, Georg-August University of Göttingen,

Microbial communities are major drivers of global elemental cycles in the oceans due to their high abundance and enormous taxonomic and functional diversity. Recent studies assessed microbial taxonomic and functional biogeography in global oceans but microbial functional biogeography remains poorly studied. Here we show that in the near-surface Atlantic and Southern Ocean between 62°S and 47°N microbial communities exhibit distinct taxonomic and functional adaptations to regional environmental conditions. Richness and diversity showed maxima around 40° latitude and intermediate temperatures, especially in functional genes (KEGG-orthologues, KOs) and gene profiles. A cluster analysis yielded three clusters of KOs but five clusters of genes differing in the abundance of genes involved in nutrient and energy acquisition. Gene profiles showed much higher distance-decay rates than KO and taxonomic profiles. Biotic factors were identified as highly influential in explaining the observed patterns in the functional profiles, whereas temperature and biogeographic province mainly explained the observed taxonomic patterns. Our results thus indicate fine-tuned genetic adaptions of microbial communities to regional biotic and environmental conditions in the Atlantic and Southern Ocean.

Selection, drift and community interactions shape microbial biogeographic patterns in the Pacific Ocean

Felix Milke[1], Irene Wagner-Döbler[2], Gerrit Wienhausen[1], Meinhard Simon[1]
[1] ICBM, University of Oldenburg
[2] Institute for Microbiology; University of Braunschweig, Braunschweig

Biogeographic patterns, known for plants and animals, have been found more recently for microorganisms as well, mainly due to the advancements in high throughput sequencing of phylogenetic marker genes and metagenomic shotgun analyses. Despite accumulating data on microbial biogeographic patterns in terrestrial and aquatic environments, we still lack a comprehensive understanding of how these patterns establish, in particular in ocean basins. Here we show the relative significance of the ecological mechanisms selection, dispersal and drift for shaping the composition of microbial communities in the Pacific Ocean over a transect of 12,400 km between subantarctic and subarctic regions. In the epipelagic, homogeneous selection contributes 50-60% and drift least to the three mechanism for the assembly of prokaryotic communities whereas in the upper mesopelagic, drift is the relatively most important mechanism for the particle-associated subcommunities. For eukaryotes >8 µm, homogeneous selection is also the most important mechanisms at two epipelagic depths whereas at all other depths drift is the predominant mechanism. As species interactions are essential for structuring microbial communities we further analysed co-occurrence based community metrics to assess biogeographic patterns over the transect. These features explained much better variations in microbial community composition as a function of abiotic and biotic variables than geographic or phylogenetic distance measures. Our analyses are important to better understand assembly processes of microbial communities in the upper layers of the largest ocean and how they adapt to effectively perform in global biogeochemical processes. Similar principles presumably act upon microbial community assembly in other ocean basins.

Distinct glycoconjugate cell surface structures make the pelagic diatom *Thalassiosira rotula* an attractive habitat for bacteria

Tran Quoc Den[1], Thomas R. Neu[2], Sabiha Sultana[1], Helge-A. Giebel[1], Meinhard Simon[1], Sara Billerbeck[1]
[1] ICBM, University of Oldenburg,
[2] Helmholtz Centre for Environmental Research – UFZ, Magdeburg, Germany

Interactions between marine diatoms and bacteria have been studied for decades. However, the visualization of physical interactions between these diatoms and their colonizers is still limited. To enhance our understanding of these specific interactions, a new *Thalassiosira rotula* isolate from the North Sea (strain 8673) was characterized by scanning electron microscopy. To investigate defined interactions of this strain with bacteria it was made axenic. The axenic strain was co-cultivated with a natural bacterial community and in two- or three-partner consortia with different bacteria of the *Roseobacter* group, *Gammaproteobacteria* and *Bacteroidetes*. The consortia were further analyzed using confocal laser scanning microscopy after staining with fluorescently labelled lectins. A screening of 78 different lectins identified six as very suitable to characterize glycoconjugates of *T. rotula*. The resulting images show that fucose-containing threads were the dominant glycoconjugates secreted by the *T. rotula* cells but chitin and to a lesser extent other glycoconjugates were also identified. Bacteria attached predominantly to the fucose glycoconjugates. The colonizing bacteria showed various attachment patterns such as adhering to the diatom threads in aggregates only or attaching to both the surfaces and the threads of the diatom. Interestingly the colonization patterns of single bacteria differed strikingly from those of bacterial co-cultures, indicating that interactions between two bacterial species impacted the colonization of the diatom. Our observations help to better understand physical interactions and specific colonization patterns of distinct bacterial mono- and co-cultures with an abundant diatom of costal seas.

A2

Auxiliary metabolic gene functions in pelagic and benthic viruses of the Baltic Sea

Benedikt Heyerhoff, Bert Engelen, Carina Bunse
ICBM, University of Oldenburg

Marine microbial communities are facing various ecosystem fluctuations (e.g. temperature, organic matter concentration, salinity or redox regimes) and thus have to be highly adaptive. This might be supported by the acquisition of auxiliary metabolic genes (AMGs) originating from virus infections. Marine bacteriophages frequently contain AMGs, which allow them to augment their host‘s metabolism or enhance virus fitness. These genes encode for the same metabolic functions as their highly similar host homologues. Here, we analyzed the diversity, distribution, and composition of marine viruses, focusing on AMGs to identify their putative ecologic role. We analyzed viruses and assemblies of 212 publicly available metagenomes obtained from sediment and water samples across the Baltic Sea. In general, the virus composition in both compartments differed compositionally. While the predominant viral lifestyle was found to be lytic, lysogeny was more prevalent in sediments than in the pelagic samples. The highest proportion of AMGs were identified in genomes of the *Myoviridae*. Overall, the most abundantly occurring AMGs encoded for functions that protect viruses from degradation by their hosts, such as methylases. Additionally, some detected AMGs are known to be involved in photosynthesis, 7-cyano7-deazaguanine synthesis and cobalamin biosynthesis among other functions. Several AMGs that were identified in this study were previously detected in a large-scale analysis including metagenomes from various origins, i.e. different marine sites, wastewater and the human gut. This supports the theory of globally conserved core AMGs that are spread over virus genomes, regardless of host or environment.

DMS from DMSO reduction fuels methanogenesis in salt marsh sediments

Dennis Alexander Tebbe[1], Charlotte Gruender[2], Martin Könneke[1], Bert Engelen[1] and Hendrik Schäfer [2]
[1] ICBM, University of Oldenburg,
[2] School of Life Sciences, University of Warwick, UK

Volatile organic compounds play a key role in global sulfur cycling. One of these compounds, dimethyl sulfide (DMS), complements this cycle via atmospheric transport from marine to terrestrial environments and is an important factor in climate regulation. Despite its global relevance, we still lack a complete understanding of its sources and sinks. This study aims to investigate the reduction of dimethyl sulfoxide (DMSO), the fate of the subsequently produced DMS and to identify the prokaryotes involved in these processes. Therefore, we performed DMSO incubation experiments in sediment slurries from salt marshes, known as hotspots for organosulfur cycling. The anaerobic slurries showed instant DMSO reduction with peak DMS concentrations after ~6 days of incubation. This was accompanied by increases in methanogenesis from DMS, leading to its complete depletion (~8 days). 16S-rRNA gene sequence analysis enrichments of members of *Oceanospirillales*, *Rhodobacterales*, SAR324, *Fusobacteriales*, *Desulfobulbales*, *Desulfobacterales* and *Kiritimatiellales* with increasing DMS concentrations and thus indicates their relation to DMSO reduction. Methanogenesis from DMS was accompanied by increased abundances of methanogens affiliated with *Methanosarciniales* (up to 46%). Our experiments showed, that DMS from DMSO reduction can fuel methanogenesis and thereby contribute significantly to methane emissions from salt marsh sediments. Ongoing investigations of 24 metagenomes from incubations and from natural salt marsh sediments aim to identify potential genes involved in cycling of organosulfur compounds. Comparisons of incubations with natural sites will enable us to better understand the metabolic functioning of identified DMSO reducers and confirm DMSO reduction as significant source of atmospheric DMS and methane.

A5

The astonishing wealth of RepABC-type plasmids in *Rhodobacterales*

Lukas Birmes, Henner Brinkmann, Heike Freese, Jörn Petersen
Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig

Background: Plasmids are supposed to play an important role in the ecological niching and evolution of *Rhodobacterales* by means of horizontal gene transfer (HGT). Low copy number RepABC plasmids are the most important ECRs of *Alphaproteobacteria*. Phylogenetic analyses of RepC replication proteins allowed to distinguish between nine different compatibility groups in *Rhodobacterales* (Petersen et al., 2009), but their stable maintenance has never been experimentally confirmed.
Objectives
(1) Proportion of RepABC replicons in the plasmid pool of *Rhodobacterales*
(2) *In silico* detection of new compatibility groups
(3) Experimental validation of plasmid compatibility
(4) Investigation of plasmid stability and host range
Methods
(1) Identification of replicase (*repC*), partitioning genes (*parAB*), palindrome sequences and type IV secretion systems (T4SSs) with BLAST searches
(2) Phylogenetic analyses of RepA, RepB & RepC proteins
(3) Cloning of *repABC* modules, functionality and compatibility test in *Phaeobacter inhibens* DSM 17395
(4) Host range test in *Agrobacterium tumefaciens* C58
Results: The investigation of 306 *Rhodobacterales* genomes revealed in total 496 RepABC, 147 DnaA-like, 139 RepB, 121 RepA and 14 RepC_soli replication systems. More than 90% of the identified T4SSs are located on RepABC-type plasmids. Phylogenetic analyses revealed the presence of at least 19 compatibility groups of RepABC-type plasmids. Experimental testing showed (i) the stable co-existence of all plasmids from different compatibility groups and (ii) the incompatibility of plasmids of the same compatibility group, which is in agreement with the distribution of diagnostic palindromes.
Conclusion: Our genome analyses showed that RepABC-type plasmids are the by far most abundant replicon type of *Rhodobacterales* while the wealth of T4SSs reflects the crucial role of RepABC-type plasmids for HGT and rapid adaptations in the ocean. The understanding of diversity, compatibility and host range of RepABC replicons provides the promising perspective to develop novel cloning vectors that are functional in *Rhodobacterales* and rhizobia.

Metagenomic insights into the marine mat-forming cyanosphere – *Coleofasciculus* and associated heterotrophic bacteria

Pia Marter, Sixing Huang, Heike M. Freese, Henner Brinkmann Jörn Petersen
Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig

Introduction: The filamentous cyanobacterium *Coleofasciculus chthonoplastes* is an ecological key player of microbial mats in tidal flats. In the coastal environment it lives in close relationship with many heterotrophic bacteria. Isolates from all over the world have been deposited at the DSMZ, which provides the promising opportunity to compare the bacterial composition of the cyanosphere.
Objectives: Complete 16S-rRNA gene sequences of *C. chthonoplastes* and the associated heterotrophic bacteria should be established and analysed based on a novel 16S-ITS-amplicon PacBio sequencing approach. Metagenome sequencing should provide substantial insights into the actual microbial composition of the cyanosphere.
Methods: 16S-ITS-amplicon and metagenome sequencing was performed on the PacBio and Illumina NovaSeq platform, respectively. For both sequencing methods, newly established

bioinformatic pipelines with filtering, clustering/binning and quality checks were used (Marter et al., 2021).
Results: The established 16S-ITS-sequences allowed us to validate the authenticity of 36 *Coleofasciculus* strains. Over 300 unique 16S-ITS sequences variants of heterotrophic bacteria were identified in the marine cyanosphere. Metagenome sequencing and binning resulted in high quality metagenome-assembled genomes (MAGs) of 14 *Coleofasciculus* strains and 306 associated heterotrophs. The number of heterotrophs within the cyanosphere varied between two and 39 high-quality MAGs.
Phylogenomic analyses showed that the genus *Coleofasciculus* forms two separate clades that might represent seven different species. Strains of clade B were only found in the North and Baltic Sea. *Marinovum algicola* (*Rhodobacterales*) is the most common housemate of *Coleofasciculus* and found in 12/14 metagenomes, followed by *Roseitalea porphyridii* (*Hyphomicrobiales*; 9/14) and *Balneola alkaliphila* (*Balneolales*; 8/14). The most promising MAG is G1-WW12-26 representing a bacterium of the candidate phylum Sumerlaeota.
Conclusion: Metagenome-sequencing provided highly resolved insights into the evolution of the genus *Coleofasciculus* and the composition of the cyanosphere. The identification of a Sumerlaeota MAG opens the opportunity to cultivate bacteria of the microbial dark matter.

A6

Phylogenomics & functional genomics of the Roseobacter clade

Jan Meier-Kolthoff, Markus Göker
Department of Bioinformatics and Databases, Leibniz Institute DSMZ

During the three funding periods project A6 successfully developed bioinformatic methods and tools for solving phylogenomic and taxonomic questions and applied them to large-scale datasets such as the entire class *Alphaproteobacteria* [6] or smaller focus groups within the *Rhodobacteraceae* [1]. Scientific highlights include (i) the highly-cited Type (Strain) Genome Server (TYGS; https://tygs.dsmz.de) [3,5], an automated high-throughput platform for state-of-the-art genome-based taxonomy and (ii) the highly-cited Virus Classification and Tree Building Online Resource (VICTOR; https://victor.dsmz.de) [4], a web service for the genome-based phylogeny and classification of (roseo-)phages. The sequencing of type-strain genomes and subsequent genome-based reclassification within the class *Alphaproteobacteria* (1,107 strains) was a major milestone, as it substantially improved the classification, which is fundamental for countless research questions across the CRC and beyond [6]. Moreover, focused analyses of the *Rhodobacteraceae* confirmed that there is no unequivocal evidence for the monophyly of roseobacters, revealed evolutionary adaptation to marine and non-marine habitats and yielded indications for several shifts between marine and non-marine habitats that occurred independently [2].

References:

1. Breider, S. et al. Genome-scale data suggest reclassifications in the Leisingera-*Phaeobacter* cluster including proposals for *Sedimentitalea* gen. nov. and *Pseudophaeobacter* gen. nov. Front. Microbiol. 5, (2014).
2. Simon, M. et al. Phylogenomics of *Rhodobacteraceae* reveals evolutionary adaptation to marine and non-marine habitats. ISME J 11, 1483–1499 (2017).
3. Meier-Kolthoff, JP. & Göker, M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10, 2182 (2019).
4. Meier-Kolthoff, JP. & Göker, M. VICTOR: genome-based phylogeny and classification of prokaryotic viruses. Bioinformatics 33, 3396–3404 (2017).
5. Meier-Kolthoff, JP. et al. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Research 50, D801–D807 (2022).
6. Hördt, A. et al. Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of *Alphaproteobacteria*. Front. Microbiol. 11, 468 (2020).

A8

Vitamin B_{12} is not shared by all marine prototrophic bacteria with their environment

Sabiha Sultana, Stefan Bruns, Heinz Wilkes, Meinhard Simon, Gerrit Wienhausen
ICBM, University of Oldenburg,

Vitamin B_{12} (cobalamin, herein B_{12}) is an essential cofactor involved in amino acid synthesis and carbon resupply to the TCA cycle for most prokaryotes, eukaryotic microorganisms, and animals. Despite being required by most, B_{12} is produced by only a minor fraction of prokaryotes and therefore leads to complex interaction between prototrophs and auxotrophs. However, it is unknown how B_{12} is provided by prototrophs to auxotrophs. In this study, B_{12} prototrophic alpha- and gammaproteobacterial strains were grown in co-culture with Thalassiosira pseudonana, a B_{12} auxotrophic diatom, to determine the bacterial ability to support the growth of the diatom by sharing B_{12}. Among these strains, 18 were identified to share B_{12} with the diatom, while 15 were identified to retain B_{12} and not support growth of the diatom. The other bacteria either shared B_{12} with the diatom only with the addition of substrate or inhibited the growth of the diatom. Extracellular B_{12} measurements of B_{12}-provider and B_{12}-retainer strains confirmed that the cofactor could only be detected in the environment of the tested B_{12}-provider strains. Intracellular B_{12} was measured by LC-MS and showed that the concentrations of the different B_{12}-provider as well as B_{12}-retainer strains differed substantially. Although B_{12} is essential for the vast majority of organisms, mechanisms that export this essential cofactor are still unknown. Our results suggest for the first time that a large number of the few bacteria that can synthesise B_{12} de novo cannot share the cofactor with their environment.

B2

Bacterial siderophore biosynthesis cluster traveling across genomes and environments

Martine Berger[1], Kathrin Schäfer[1], Jan Tebben[2,3], Jan Meier-Kolthoff[4], Tilmann Harder[2], Markus Göker[4] Thorsten Brinkhoff[1]

[1] ICBM, University of Oldenburg,
[2] Institute of Marine Chemistry, University of Bremen
[3] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[4] Department of Bioinformatics and Databases, Leibniz Institute DSMZ

Iron is an essential micronutrient for bacterial growth, but for aerobic bacteria acquisition of iron is a particular challenge due to the poor bioavailability in oxic environments. To cope with iron limitation, the production of siderophores, high-affinity iron chelators, is one important iron acquisition strategy. The siderophore petrobactin, encodes by the *asbABCDEF* operon, is vital for iron acquisition and full virulence in *Bacillus anthracis*. *We show that the asb* operon is widespread over three classes, *Firmicutes*, α- and γ-*Proteobacteria,* ranging from members of the *Bacillus cereus* group, which are well-known opportunistic human pathogens causing foodborne illnesses to plant growth promoting bacteria up to marine *Rhodobacterales* often associated with eukaryotes in a mutualistic lifestyle. Phylogenetic analysis suggests that *the asb* operon was transferred horizontally across different bacterial classes, potentially driven by the pressure to compete for iron. Thereby, the *asb* cluster may experience diversifying selection that alters the structure of petrobactin yielding roseobactin and rhodobactin. Gene fusion is one further important mechanism concurred, such as the fusion of *asbD* and *asbE* as well as *asbB* and *asbC*, which could be a reason for the enhanced performance in *Paracoccus*. The spare and patchy distribution of the *asb* cluster could be explained by extensive loss events during evolution, whereby the receptor for uptake kept. This results in a 4-fold higher

number of species harbouring only the receptor within the *Rhodobacterales*, which allows the bacteria to take up foreign siderophores without costly production.

Response of a marine diatom to a bacterial signalling molecule

Sujatha Srinivas[1], Dmytro Spriahailo[1], Anja Poehlein[2], Rolf Daniel[2], Katharina Hoff[3], Tilmann Harder[4], Jan Tebben[4,5], Uwe John[5,6], Thorsten Brinkhoff[1]

[1] Institute for Chemistry and Biology of the Marine Environment, University of Oldenburg, Germany
[2] Genomic and Applied Microbiology and Göttingen Genomics Laboratory, University of Göttingen, Germany
[3] Institute for Mathematics and Informatics, University of Greifswald, Germany
[4] Marine Chemistry, University of Bremen, Germany
[5] Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany
[6] Helmholtz Institute for Functional Marine Biodiversity, University of Oldenburg, Germany

Tropodithietic acid (TDA), a broad spectrum antibiotic, is produced by a subgroup of marine bacteria of the family *Rhodobacteraceae*, i.e. members of the genera *Phaeobacter*, *Ruegeria* and *Pseudovibrio*. In some of its producers, TDA at sub-inhibitory concentrations, acts as a signalling molecule and chemoeffector, thus exhibiting dual functionality. The TDA-producing strain *Phaeobacter inhibens* DSM 17395 is a prolific biofilm former and was found in association with various marine eukaryotes, e.g., the ubiquitous diatom *Thalassiosira rotula*. Mesocosm studies have shown that in colonization experiments DSM 17395 accounts for a major fraction of the early bacterial community on *T. rotula*. To investigate a potential role of TDA as an inter-kingdom signalling molecule, we tested different TDA concentrations on *T. rotula* cultures and analyzed growth, transcriptomic and metabolomic responses at mid-exponential and early stationary phase. We performed Illumina sequencing and de novo transcriptome assembly of high-quality reads into 22,420 genes and subjected them to BLAST alignment, Gene Ontology and KEGG Orthology annotation. In presence of 1µM TDA, growth of the diatom was two-fold higher and 588 genes were found to be significantly differentially expressed. Metabolomic analysis showed that TDA was taken up from medium into the cellular fraction within 6 hours after addition and subsequently decreased in both fractions. Ongoing analysis of the differentially expressed genes and metabolites will elucidate the nature of the diatom's response to TDA and its role as a bacterial signalling molecule in diatom-bacteria interaction.

B7

Sulfur Metabolism in Marine Bacteria

Anuj Chhalodia and Jeroen S. Dickschat

Kekulé-Institute for Organic Chemistry and Biochemistry, University of Bonn

Marine bacteria from the *Roseobacter* clade have an extensive sulfur metabolism with great importance for marine and global ecosystems.[1] One of the main sulfur metabolites is represented by dimethylsulfoniopropionate (DMSP), which is formed by algae and bacteria. Its enzymatic degradation proceeds through two pathways, i. e. the lysis pathway (cleavage to dimethylsulfide and acrylate) and the demethylation pathway leading to MeSH.[2,3] We have tested several DMSP lyases for their potential to convert DMSP analogues into sulfur volatiles, including diallylsulfoniopropionate (DAllSP) and allylmethylsulfoniopropionate (AllMSP) (Figure 1A).[4-6] Interestingly, these DMSP analogs result in the formation of the garlic odour constituents diallyldisulfide and diallyltrisulfide.[6] Recently also an oxidised DMSP metabolite, dimethylsulfoxoniumpropionate (DMSOP), was discovered, but so far no enzymes for its degradation have been reported.[7] We have tested several DMSP lyases for their potential to cleave DMSOP into DMSO and acrylate (Figure 1B). The results of this work will be presented on the poster.

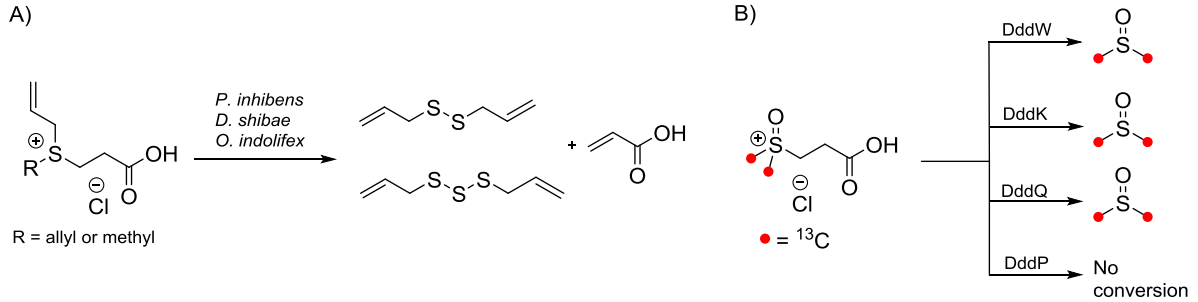


Figure 1. A) Formation of garlic odour constituents from DAllSP and AllMSP. B) DMSOP degradation by DMSP lyases.

References

[1] S. J. Giovannoni, U. Stingl, *Nature* **2005**, *437*, 343.
[2] J. D. Todd, R. Rachel, Y. G. Li, W. Margaret, L. B. Philip, S. Lei, R. J. C. Andrew, M. Gill, S. Michael, A. W. B. Johnston, *Science* **2007**, *315*, 666.
[3] E. C. Howard, J. R. Henriksen, A. Buchan, C. R. Reisch, H. Bürgmann, R. Welsh, W. Ye, J. M. Gonzalez, K. Mace, S. B. Joye, R. P. Kiene, W. B. Whitman, M. A. Moran, *Science* **2006**, *314*, 649.
[4] J. S. Dickschat, C. Zell, N. L. Brock, *ChemBioChem* **2010**, *11*, 417.
[5] I. Burkhardt, L. Lauterbach, N. L. Brock, J. S. Dickschat, *Org. Biomol. Chem.* **2017**, *15*, 4432.
[6] A. K. Chhalodia, J. S. Dickschat, *Beilstein J. Org. Chem.* **2021**, *17*, 569.
[7] K. Thume, B. Gebser, L. Chen, N. Meyer, D. J. Kieber, G. Pohnert, *Nature* **2018**, *563*, 412.

C5

The role of biotin for the interaction between *Dinoroseobacter shibae* and *Prorocentrum cordatum*

Johannes Mansky, Irene Wagner-Döbler
Institute for Microbiology; University of Braunschweig, Braunschweig

The red-tide dinoflagellate *P. cordatum* and the roseobacter model organism *Dinoroseobacter shibae* shift from a mutualistic to a pathogenic phase during co-cultivation. The killing phenotype is determined by the 191 kb plasmid and can be conjugated into other roseobacters. From a transposon-library of *D. shibae* we retrieved 28 mutants whose insertion sites were located on the 191 kb plasmid. We co-cultivated each of them with *P. cordatum* in L1 medium lacking vitamin B12. With three transposon mutant strains, the initial symbiotic phase was intact but the later pathogenic phase was lost in co-culture. The mutations were located in an operon predicted to encode genes for biotin (B7) uptake, including an ABC transporter and the BioY protein. Since both *P. cordatum* and *D. shibae* are auxotrophic for biotin, we hypothesized that the bacterium depletes the medium from biotin resulting in apoptosis of the dinoflagellate. We then performed dual RNAseq of co-cultures of dinoflagellate and *D. shibae* wild-type as well as mutants of *D. shibae* lacking the transporters for uptake of B7 showing the details of this interaction at different biotin concentrations.

C7

Bioenergetics modelling of bacterial growth on a mixture of organic resources

Leonhard Lücken and Bernd Blasius

We develop a bioenergetic model framework to describe a population of heterotrophic marine bacteria growing on a mixture of organic resources. Here we compare three different models (I-III) focussing on the reproduction of diauxic shifts observed in a batch culture of the marine bacterium Phaeobacter Inhibens. Each model captures the temporal dynamics of bacterial growth and of depletion of carbon sources (sugars and amino acids).

Z02

Latitudinal and depth gradients along the Pacific Ocean: Bacterial and archaeal core communities

Jacqueline Hollensteiner[1], Bernd Wemheuer[1], Helge-A. Giebel[1], Meinhard Simon[1], Rolf Daniel[1]
[1] Genomic and Applied Microbiology and Göttingen Genomics Laboratory, University of Göttingen, Germany
[2] ICBM, University of Oldenburg,

The Pacific Ocean is one of the largest and least studied changing oceanic regions, divided into 56 biogeochemical Longhurst provinces. Marine microbial communities, particularly bacteria and archaea, are highly diverse and essential for ecosystem stability and climate maintenance, as they participate in the biological pump.
In recent years, many studies have been conducted on the composition of microbial communities in the oceans. However, most studies are limited to at least some aspects, as the size of the ocean itself is a major challenge. Given the importance of microbial communities and their contribution to biogeochemical cycles in the marine environment, the need for new studies covering larger contiguous transects, combined with a comprehensive collection of metadata for robust interpretation, is obvious.
Here, we conducted 16S rRNA sequencing of metagenomic material from FL and PA seawater filters from a Pacific Ocean transect along the 180° meridian from sub-Antarctic waters at 52°S to the Bering Sea at 59°N. It covers 25 stations, three depths and 11 Longhurst provinces.
Microbial taxa generally followed one of three distinct abundance patterns: i) near the equator and at lower latitudes, ii) at higher latitudes, or iii) at northern higher latitudes. Bacterial key taxa include *Cyanobiaceae, Flavobacteriaceae*, SAR11 Clade I, *Rhodobacteraceae,* and *Actinobacteriacae*. For archaea Marine Group II and III were most abundant. Microbial communities shift according to Longhurst provinces, regardless of lifestyle. The most important factors for this effect were oxygen, temperature, and salinity. Alpha diversity and richness showed a consistent latitudinal pattern along the Pacific Ocean, peaking near the equator decreasing with depth at higher latitudes. Microbial richness and diversity in the PA fraction were significantly higher compared to FL.
This study provides new insights into the bacterial and archaeal communities along a Pacific latitude and their impacts.