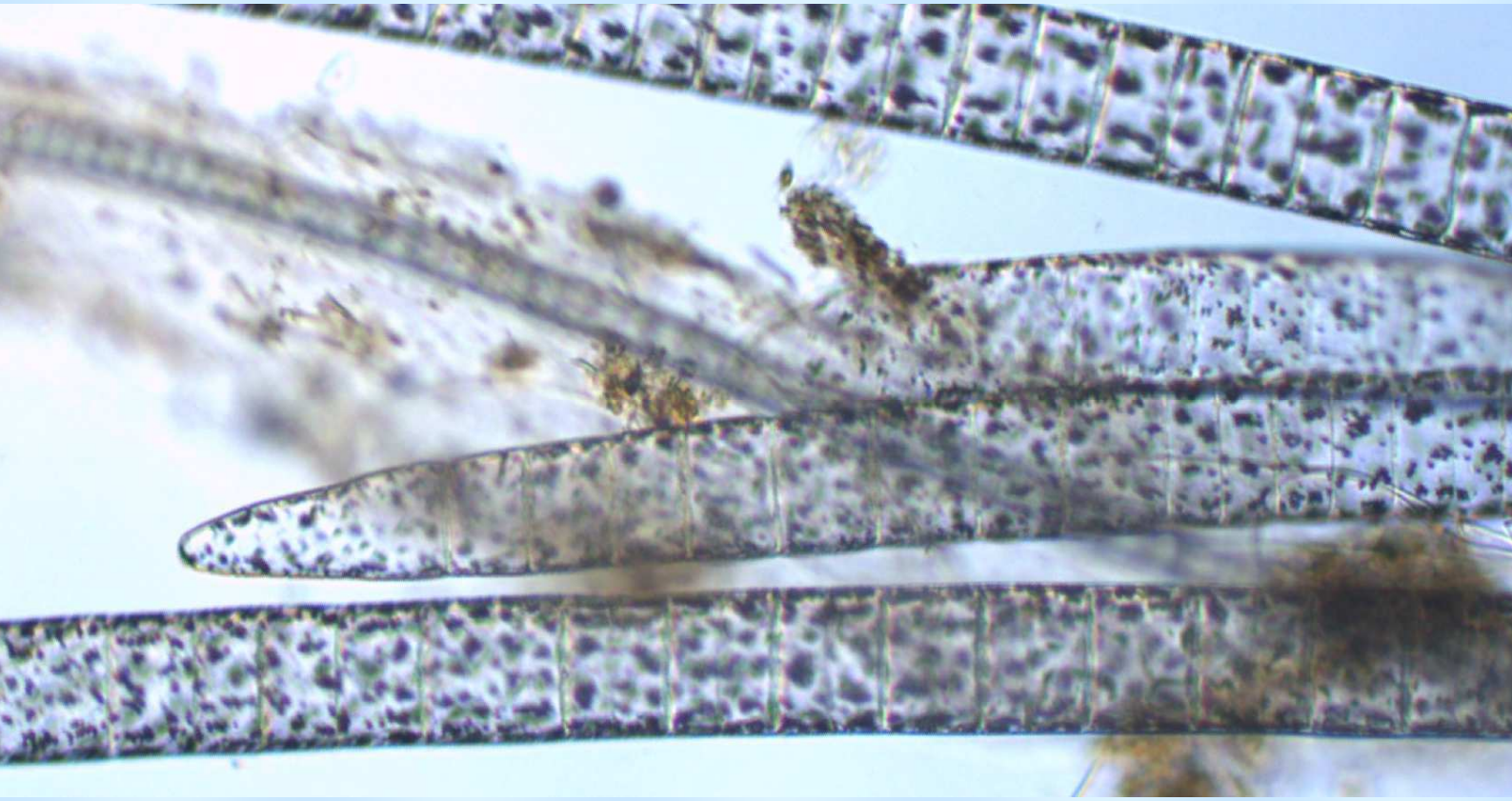
EMBO Workshop on Microbial Sulfur Metabolism

12–15 April 2015

Helsingør, Denmark

Program and abstracts



Thioploca sp., South America

Workshop Organization

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Niels-Ulrik Frigaard, University of Copenhagen, Denmark

Scientific Committee

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Christiane Dahl, University of Bonn, Germany
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Gerard Muyzer, University of Amsterdam, The Netherlands
Inês A. C. Pereira, New University of Lisbon, Portugal

Venue

Konventum
Gl. Hellebækvej 70
3000 Helsingør
Denmark

http://www.konventum.dk/

Sponsors

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Program

Sunday, 12 April 2015

From 14:00	Check-in at conference center
16:00-18:00	Registration Poster mounting Coffee in poster area
18:00-19:00	Dinner
19:00-19:55	**Welcome and Key Note Lecture** Chairs: Kai Finster and Niels-Ulrik Frigaard
19:00-19:15	Welcome
19:15-19:55	*Key note lecture:* **Lars Peter Nielsen**, Denmark (L1) Electrogenic sulfur oxidation by cable bacteria in sediments and soils
20:00-22:00	Mixer in "Salonerne"
Until 1:00	Bar open

Monday, 13 April 2015

7:00-8:30	Breakfast
8:30-10:00	**Session 1: Biogeochemistry (Part 1 of 2)** Chair: Filip Meysman
8:30-9:00	*Invited lecture:* **David Johnston**, USA (L2) The information locked in the oxygen isotope composition of sulfate
9:00-9:20	**Boswell Wing**, Canada (L3) Sulfur isotope discrimination during microbial sulfate respiration: linking biogeochemical signals to biochemical and physiological state
9:20-9:40	**Marion Jaussi**, Denmark (L4) Sulfate reduction rates and the size of the sulfate-reducer community are tightly linked in the subsurface of an arctic fjord (Greenland)
9:40-10:00	**Hannah Sophia Weber**, Denmark (L5) Sulfur cycling and isotope fractionation coupled to anaerobic methane oxidation in a low sulfate environment
10:00-10:30	Coffee break

10:30-11:40	**Session 1: Biogeochemistry (Part 2 of 2)** Chair: David Johnston
10:30-11:00	*Invited lecture:* **Donald E. Canfield**, Denmark (L6) Interpreting sulfur isotope signatures, progress and problems
11:00-11:20	**Filip Meysman**, Netherlands (L7) Electrical currents and cryptic sulphur cycling in coastal sediments
11:20-11:40	**Nils Risgaard-Petersen**, Denmark (L8) Cable Bacteria in Freshwater Sediments
12:00-13:00	Lunch
14:00-15:40	**Session 2: Ecology and Evolution (Part 1 of 2)** Chair: Casey Hubert
14:00-14:30	*Invited lecture:* **Heide Schulz-Vogt**, Germany (L9) Large sulfur bacteria
14:30-15:00	*Invited lecture:* **Karthik Anantharaman**, USA (L10) Bacterial sulfur oxidation genes in deep-sea viruses
15:00-15:20	**Hendrik Schaefer**, United Kingdom (L11) Characterization of uncultured dimethylsulfide degrading gammaproteobacteria from a coastal saltmarsh using stable isotope probing and single cell genomics
15:20-15:40	**Gerard Muyzer**, Netherlands (L12) Ecogenomics of haloalkaliphilic sulphur bacteria
15:40-16:10	Coffee break
16:10-17:40	**Session 2: Ecology and Evolution (Part 2 of 2)** Chair: Heide Schulz-Vogt
16:10-16:40	*Invited lecture:* **Casey Hubert**, Canada (L13) Thermospores of sulfate-reducing bacteria as biogeographic indicators
16:40-17:00	**Bela Hausmann**, Austria (L14) The power of the rare: Sulfate reduction in an acidic peatland is driven by small networks of natively low abundant bacteria
17:00-17:20	**Kasper Urup Kjeldsen**, Denmark (L15) Can cable bacteria actually oxidize sulfide? Insights from a genome
17:20-17:40	**Vera Thiel**, USA (L16) Metagenomic study reveals first sulfate-reducing member of the Bacteroidetes-Chlorobi group
18:00-19:00	Dinner
19:00-21:00	**Posters and coffee** (even numbered posters presented)
Until 1:00	Bar open

Tuesday, 14 April 2015

7:00-8:30	Breakfast
8:30-10:00	**Session 3: Students' Contest** Chair: Kai Finster
8:30-8:45	**Stefan Dyksma**, Germany (L17) Ubiquitous Gammaproteobacteria dominate dark carbon fixation in coastal sediments
8:45-9:00	**Xiaofen Wu**, Sweden (L18) Metagenomes from the terrestrial deep biosphere reveal sulfur oxidation and reduction pathways
9:00-9:15	**Petra Henke**, Germany (L19) Tight symbiotic interactions in the pelagic sulfur cycle - the case of phototrophic consortia
9:15-9:30	**Jasmine Berg**, Germany (L20) Single-cell investigations into the metabolism of stored sulfur in living bacteria
9:30-9:45	**Diana Vasquez Cardenas**, Netherlands (L21) Microbial carbon metabolism associated with electrogenic sulphur oxidation in coastal sediments
9:45-10:00	**Jon Graf**, Germany (L22) Metagenome and mRNA expression analysis of the bacterial partner of an AOM-mediating microbial consortium
10:00-10:30	Coffee break
10:30-12:00	**Session 4: Physiology and Biochemistry (Part 1 of 2)** Chair: Ulrike Kappler
10:30-11:00	*Invited lecture:* **Inês A. C. Pereira**, Portugal (L23) What is the physiological product of the DsrAB dissimilatory sulfite reductase?
11:00-11:20	**Sofia Venceslau**, Portugal (L24) New insights into the energy metabolism of *Desulfovibrio vulgaris*: The role of FlxABCD-HdrABC, a novel NADH dehydrogenase-heterodisulfide reductase
11:20-11:40	**André Santos**, Portugal (L25) Crucial role of DsrC in dissimilatory sulfite reduction
11:40-12:00	**Rich Boden**, United Kingdom (L26) Chemolithoheterotrophy – new insights into an often forgotten yet widespread metabolic trait
12:00-13:00	Lunch

13:15-14:55	**Session 4: Physiology and Biochemistry (Part 2 of 2)** Chair: Inês A. C. Pereira
13:15-13:45	*Invited lecture:* **Christiane Dahl**, Germany (L27) An integrated view on sulfur oxidation in purple sulfur bacteria
13:45-14:15	*Invited lecture:* **Jillian Petersen**, Germany (L28) Sulfur-oxidizing symbionts: novel pathways and novel organisms
14:15-14:35	**Marianne Guiral**, France (L29) Oxidation of thiosulfate and sulfite by the hyperthermophilic bacterium *Aquifex aeolicus*
14:35-14:55	**Ulrike Kappler**, Australia (L30) Organosulfur compound metabolism in the human pathogen *Haemophilus influenzae*
15:00-17:00	**Posters and coffee** (odd numbered posters presented)
17:15-19:00	**Excursion to Kronborg Castle**
20:00-22:00	Conference Dinner
Until 1:00	Bar open

Wednesday, 15 April 2015

Before 9:00	Check-out of conference center
7:00-8:00	Breakfast
8:00-9:10	**Session 5: Sulfur Transformations** Chair: Rich Boden
8:00-8:30	*Invited lecture:* **Silke Leimkühler**, Germany (L31) Biosynthesis of the molybdenum cofactor and its relation to sulfur metabolism
8:30-8:50	**Tom Berben**, Netherlands (L32) Comparative metabolic studies of the halo-alkaliphilic chemolithoautotrophic sulfur-oxidizing bacterium *Thioalkalivibrio thiocyanoxidans* ARh 2
8:50-9:10	**Marc Mussmann**, Germany (L33) Ecology and ecogenomics of uncultured sulfate-reducing bacteria ubiquitous and abundant in marine sediments
9:10-9:40	Coffee break

9:40-11:20	**Session 6: Biotechnology** Chair: Christiane Dahl
9:40-10:10	*Invited lecture:* **Piet Lens**, Netherlands (L34) Applications of the biological sulfur and selenium cycles in environmental biotechnology
10:10-10:40	*Invited lecture:* **Ian Head**, United Kingdom (L35) Sulfur-metabolizing microbes in oil degradation and corrosion
10:40-11:00	**Barrie Johnson**, United Kingdom (L36) Development and application of acidophilic sulfidogenic bioreactors for combined pH amelioration, sulfate removal and selective recovery of metals from acidic waste waters
11:00-11:20	**Mark Dopson**, Sweden (L37) Oxidation of inorganic sulfur compounds in metal sulfide processing wastewaters generates an electrical current in microbial fuel cells
11:20-11:35	**Poster and oral presentation awards** Chair: Kai Finster
12:00-13:00	Lunch
13:00-14:20	**Session 7: Microbial Interactions and Environmental Impacts** Chair: Marc Mussmann
13:00-13:30	*Invited lecture:* **David Schleheck**, Germany (L38) Sulfoquinovose degradation pathways in bacteria
13:30-14:00	*Invited lecture:* **Jana Milucka**, Germany (L39) The role of sulfur in marine methane oxidation
14:00-14:20	**Naoki Kamiya**, Japan (L40) Sulfur disproportionation is achieved by co-metabolism with photosynthetic sulfide oxidation to sulfur
14:20-15:10	**Key Note Lecture and Closing** Chairs: Niels-Ulrik Frigaard and Kai Finster
14:20-15:00	*Key note lecture:* **H. Rex Gaskins**, USA (L41) Microbial sulfur metabolism and colorectal cancer risk
15:00-15:10	Closing
15:30	Departure

Abstracts of Lectures

Abstracts are organized according to the sequence of presentation.

Key Note Lecture

L1

Electrogenic sulfur oxidation by cable bacteria in sediments and soils

Lars Peter Nielsen

Center for Geomicrobiology, Section for Microbiology, Department of Bioscience, Aarhus University, Ny Munkegade 114, DK-8000, Aarhus C, Denmark

Spectacular behavioral, morphological, and physiological traits have evolved among the prokaryotes competing for the lucrative aerobic oxidation of sulfide in marine sediments. The most recent and surprising finding is electrical cable bacteria, which use internal conductors to mediate electron transport over centimeter distances from sulfide at depth to oxygen at the sediment surface. All sequenced cable bacteria form a cluster of multicellular filamentous bacteria within the Desulfobulbaceae family and all show a conspicuous ring of parallel, putative electric wires inside a periplasmic continuum. The spatial separation of oxidation and reduction processes in distant cells challenge much conventional thinking in sediment biogeochemistry and microbial physiology. That cable bacteria have been overlooked until now, despite their abundance and impact in many environments, further reminds us about all the unknowns out there. My lecture will summarize present knowledge about biology, occurrence, importance, electronics and diversity of cable bacteria and then discuss some of the most exciting open questions. As an example, many single-celled sulfur oxidizers seem to co-exist with cable bacteria, thus raising the possibility that cable bacteria themselves are not sulfur bacteria but rather purely electric bacteria needing the real sulfur bacteria to deliver the electrons from sulfide oxidation.

Invited Lecture

L2

The information locked in the oxygen isotope composition of sulfate

D. Johnston, A. S. Bradley, B. Cowie

Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA, USA

Our collective understanding of the modern and geological sulfur cycle benefits from the fact that microorganisms – the agents of nearly all geochemical S cycling – impart large isotope effects. Most commonly, sulfur isotopes in sulfate and sulfide (both aqueous and in mineral form) are employed to track everything from rates of microbial processes (1) through to the presence/absence of certain metabolic clades in a given environment (2, 3). In complement to sulfur isotope studies, one under-developed tool to track sulfur recycling comes from the interrogation of oxygen isotope change in sulfate reservoirs (marine, pore water, mineralogical) (4, 5). As S-O bonds are broken and formed through inorganic and biogeochemical activity, the $^{18}O/^{16}O$ composition of the related sulfate reservoir evolves. In this work we will document the extraordinary isotopic consistency across environmental $^{18}O/^{16}O$ sulfate records and outline a rigorous, mechanism-focused approach to unpacking these records.

A small but rich body of literature sets the stage for our work [see (4)]. Much of the previous work focus falls on calibrating an inorganic equilibrium between intracellular water and sulfite (SO_3^{2-}) – a large isotope effect that significantly influences the O isotope composition of sulfate (6). Other candidate isotope exchange reactions include those associated with the generation and destruction of APS, another intracellular intermediate common in both sulfate reducing bacteria and, at a minimum, sulfur disproportionating bacteria. In parallel, there is additional and complementary information gained through the inclusion of ^{17}O measurements ($^{17}O/^{16}O$). Although in its infancy, the usage of $^{17}O/^{16}O$ provides a direct test of hypotheses derived from the $^{18}O/^{16}O$ studies noted above and provide a unique quantitative glimpse into the direct linkages between the microorganisms that drive the sulfur cycle and the contemporaneous atmosphere, all of which is captured in the isotopic composition of seawater sulfate.

1. W. D. Leavitt, A. S. Bradley, I. Halevy, D. T. Johnston (2013) Influence of sulfate reduction rates on the Phanerozoic sulfur isotope record. Proc Natl Acad Sci

2. D. E. Canfield, A. Teske (1996) Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulphur-isotope studies. Nature 382, 127-132

3. D. T. Johnston, B. A. Wing, J. Farquhar, A. J. Kaufman, H. Strauss, T. W. Lyons, L. C. Kah, D. E. Canfield (2005) Active microbial sulfur disproportionation in the Mesoproterozoic. Science 310, 1477-1479

4. G. Antler, A. V. Turchyn, V. Rennie, B. Herut, O. Sivan (2013) Coupled sulfur and oxygen isotope insight into bacterial sulfate reduction in the natural environment. Geochim Cosmochim Acta 118, 98-117

5. A. V. Turchyn, D. P. Schrag (2004) Oxygen isotope constraints on the sulfur cycle over the past 10 million years. Science 303, 2004-2007

6. S. D. Wankel, A. S. Bradley, D. L. Eldridge, D. T. Johnston (2014) Determination and application of the equilibrium oxygen isotope effect between water and sulfite. Geochim Cosmochim Acta 125, 694-711

L3

Sulfur isotope discrimination during microbial sulfate respiration: linking biogeochemical signals to biochemical and physiological state

Boswell Wing[1], Itay Halevy[2], André Pellerin[1], Jyotsana Singh[1]

[1] *Department of Earth and Planetary Sciences and GEOTOP, McGill University*
[2] *Department of Earth and Planetary Sciences, Weizmann Institute of Science*

Aqueous sulfate supports widespread anoxic respiratory carbon cycling by microbes. Aqueous sulfide is produced during this process and, when it escapes re-oxidation, it can be sequestered in metallic sulfide minerals for geological timescales. The preferential consumption of ^{32}S-subsituted sulfate during dissimilatory sulfate reduction enables sulfur isotope measurements to support these inferences. Sulfate in modern sedimentary porewaters is ^{34}S enriched, while aqueous sulfide is ^{34}S depleted, providing a rational framework for monitoring the biogeochemistry of microbial sulfur cycling throughout Earth's history.

Dissimilatory sulfate reduction was isotopically characterized through culture experiments more than a half century ago. More recent work has precisely calibrated how the magnitude of sulfur isotopic discrimination covaries inversely with the average sulfate reduction rate of a microbial population and directly with sulfate levels in the local environment. At the low-rate limit, sulfate-reducing microbes preferentially process ^{34}S-substituted sulfate to a degree that approaches the isotopic partitioning defined by thermodynamic equilibrium between aqueous sulfate and sulfide. Separation of ^{34}S from ^{32}S appears to follow a strain-specific trajectory as microbes respire faster than this limit.

In this presentation, we will discuss how these gross physiological observations provide a link between isotopic constraints on biogeochemical sulfur cycling and the average biochemical state of a population of sulfate-reducing microbes. We will focus on predictions that explicitly link preferential processing of ^{34}S-enriched metabolites to enzymatic reversibility and intracellular metabolite concentrations. These will focus on, for example, the in-vivo reversibility of dissimilatory (bi)sulfite reductase, the covariation of respiratory enzyme production with intracellular sulfate reduction rate, and the energetic tradeoff between ribosome production and the survival of respiratory proteins. While the presentation may inspire dissension, we hope it will encourage discussion that links the isotopic biogeochemistry of microbial sulfur metabolisms to their biochemical and physiological state.

L4

Sulfate reduction rates and the size of the sulfate-reducer community are tightly linked in the subsurface of an arctic fjord (Greenland)

Marion Jaussi[1], Kasper Urup Kjeldsen[1], Marit-Solveig Seidenkrantz[2], Bente Aagaard Lomstein[1, 3], Bo Barker Jørgensen[1], Hans Røy[1]

[1] Center for Geomicrobiology, Department of Bioscience, Aarhus University, Ny Munkegade 114, Building 1540, DK-8000 Aarhus C, Denmark
[2] Centre for Past Climate Studies and Arctic Research Centre, Department of Geoscience, Aarhus University, Hoegh-Guldbergs Gade 2, DK-8000 Aarhus C, Denmark
[3] Department of Bioscience, Microbiology, Aarhus University, Ny Munkegade 114, Building 1540, DK-8000 Aarhus C, Denmark

Sulfate reduction contributes to a major part of the organic matter mineralization in shelf sediments both in temperate and arctic environments. So far, rates of catabolic metabolism have rarely been quantified together with community size within the same sediment samples, although this association is crucial to address the metabolic state of individual cells. We quantified sulfate reduction rates with radiotracer incubation and the community size of sulfate reducers together in a highly dynamic fjord system (South West Greenland). The four sediment cores presented intriguing and diverse depth patterns of sulfate reduction rates, followed tightly by the abundance of sulfate-reducers. The mean cell-specific sulfate reduction rates were constant with depth in spite of large changes in sulfate reduction rates. Interestingly, the level of the constant per-cell sulfate reduction rates differed from site to site.

L5

Sulfur cycling and isotope fractionation coupled to anaerobic methane oxidation in a low sulfate environment

Hannah Sophia Weber[1], Kirsten Silvia Habicht[2], Bo Thamdrup[1]

[1] Nordic Center for Earth Evolution and Department of Biology, University of Southern Denmark, Campusvej 55, 5230 Odense M, Denmark
[2] Unisense A/S, Tueager 1, 8200 Aarhus N, Denmark

Sulfate–dependent anaerobic oxidation of methane (AOM) was described as an efficient sink of the greenhouse gas methane in various marine habitats. Freshwater sediments are often low sulfate environments and only since recently, evidence is accumulating for efficient anaerobic methane consumption under extremely low sulfate conditions. Taxonomic identities of the AOM–active microorganisms in freshwater sediments and their metabolic constraints remain unknown. The present study was carried out in iron–rich Lake Ørn in Denmark, which previously showed AOM activities at sulfate concentrations as low as 3 µmol L^{-1} and a the consumption of ~90 % of the produced methane in a sulfate-methane transition zone well below the oxic realm. The AOM zone was associated with a large ^{34}S depletion in the reduced sulfur pool and a strong accumulation of zero–valent sulfur. In slurry incubations, we showed that methane addition to sediment of the AOM–active zone induced sulfate consumption and associated isotope fractionation, apparently through a kinetic isotope effect. Our results strongly suggest that AOM is capable of ^{34}S fractionation at sulfate concentrations <50 µmol L^{-1} and that sulfur isotope signatures in low-sulfate environments may be strongly impacted by AOM. Furthermore, with the aim to identify the AOM–active microorganisms, we applied RNA–stable isotope probing to AOM–active sediment of Lake Ørn incubated with ^{13}C–methane or ^{13}C–bicarbonate. 16S rRNA clone library–derived sequences revealed ^{13}C–incorporation of both substrates mainly into the environmental clade GoM Arc 1 (Euryarchaeota), whereas anaerobic methane oxidizers known from marine habitats (AMNE strains) could not be detected. This study suggests that sulfur cycling in low-sulfate freshwater environments may be impacted by anaerobic methane oxidizers with taxonomic identities and enzymatic pathways different from those found in marine habitats.

Invited Lecture

L6

Interpreting sulfur isotope signatures, progress and problems

Donald Canfield

Department of Biology, University of Southern Denmark, Odense, Denmark

The isotopic composition of sulfur species in the environment reflects interplay between biological processes that impart fractionations and physical/environmental processes that control the extent to which these fractionations will be expressed. Understanding the nature of this interplay presents a crucial challenge in interpreting sulfur isotopic compositions. In my talk I will review recent progress in our understanding of the capabilities of microbial populations to impart fractionations. Despite this progress, I will demonstrate, through several examples, many of the challenges that still exist in interpreting the sulfur isotope record as preserved in modern and ancient sediments.

L7

Electrical currents and cryptic sulphur cycling in coastal sediments

Filip Meysman[1, 2], Dorina Seitaj[1], Fatimah Sulu-Gambari[3], Regina Schauer[4], Caroline Slomp[3]

[1] Department of Ecosystem Studies, Royal Netherlands Institute for Sea Research (NIOZ), Korringaweg 7, 4401 NT Yerseke, The Netherlands
[2] Department of Analytical Environmental and Geo-Chemistry, Vrije Universiteit Brussel (VUB), Pleinlaan 2, 1050 Brussels, Belgium
[3] Department of Earth Sciences (Geochemistry), Faculty of Geosciences, Utrecht University, Budapestlaan 4, 3584 CD Utrecht, The Netherlands
[4] Department of Bioscience, Center of Geomicrobiology, Aarhus University, Ny Munkegade 116, 8000 Aarhus, Denmark

Sulfate reduction is the dominant pathway for organic matter mineralization in coastal sediments, and hence, vast amounts of free sulphide are produced in the pore water. Nonetheless, the surface layer of coastal sediments often exhibits a centimeter wide zone, where neither oxygen nor free sulphide is present, suggesting that intense cryptic sulphur (S) cycling takes place. We studied the geochemical transformations and microbial drivers associated with cryptic S cycling in the sediments of a seasonally hypoxic basin, where recently cable have been found in situ (Marine Lake Grevelingen, The Netherlands). By combining microbial and geochemical data, distinct geochemical fingerprints could be linked to specific mechanisms of suboxic zone formation, which revealed a remarkable temporal succession of different cryptic S cycling pathways. Electrogenic sulphur oxidation by cable bacteria dominated the sediment geochemistry in winter and strongly solubilized the Fe-sulphide pool, which led to the formation of a large pool of Fe-oxides in the surface sediment. In late spring, these Fe-oxides were reduced to Fe-sulfides thereby acting as a sink for sulfide, whereas after the summer hypoxic period, *Beggiatoa*-mats colonized the sediment. Our data show that internal shifts in microbial communities can induce strong seasonality in sedimentary biogeochemical cycling, independent of the seasonal variation in bottom water oxygenation.

L8

Cable bacteria in freshwater sediments

Nils Risgaard-Petersen[1], Lars Peter Nielsen[1], Lars Damgaard[2], Jesper Berg[2]

[1] Center for Geomicrobiology, Section for Microbiology, Department of Bioscience, Aarhus University, Ny Munkegade 114, DK-8000, Aarhus C, Denmark
[2] Section for Microbiology, Department of Bioscience, Aarhus University, Ny Munkegade 114, DK-8000 Aarhus C, Denmark

In marine sediments cathodic oxygen reduction at the sediment surface can be coupled to anodic sulphide oxidation in deeper anoxic layers through electrical currents mediated by filamentous, multicellular bacteria of the Desulfobulbaceae family, the so called cable bacteria. In this study we tested if the same occurs in freshwater sediments. Homogenized sediments collected from the stream Giber Å, Denmark, were incubated in the laboratory. After two weeks pH signatures and electric fields, signified that anodic and cathodic reactions had developed. In situ measurements of oxygen, pH and electric potential distributions in the sediment in waterlogged banks of Giber Å further confirmed the presence of distant electric redox coupling. Filamentous Desulfobabace, with microcable morphology were found abundantly in all cases. The results of the present study indicate that electric currents mediated by cable bacteria could be important for sulfurcycling in freshwater sediments

Invited Lecture

L9

Large sulfur bacteria

Heide Schulz-Vogt

Leibniz Institute for Baltic Sea Research Warnemuende (IOW), Germany

The family Beggiatoaceae contains several linages of giant bacteria, which have caught the curiosity of microbiologists since centuries. Locally, they can also be of high environmental importance, because of the enormous biomasses, that they can build up in environments with high sulfide fluxes. Even though the genus *Beggiatoa* was first described more than 200 years ago, the phylogenetic relationship among members of this family has only been unveiled recently. Now we have to take into account, that also in this family, members that appear to be identical, because of a very similar morphology may not be closely related and possibly very distinct in physiology. Thus, with the increasing availability of genomic information it becomes more and more important to distinguish between environmental sequences originating from loosely related linages, in order to precisely describe the ecological niches of the respective genera. Lately, we came to learn, that the physiological potential in this family is even more divers than originally thought, including the use of a mixture of electron donors and acceptors as well as the use of storage compounds such as PHB or polyphosphate. Especially the letter can have important consequences for the phosphorus cycling in marine sediments and represents a poorly investigated link between the sulfur and the phosphorus cycle.

Invited Lecture

L10

Bacterial sulfur oxidation genes in deep-sea viruses

Karthik Anantharaman

Earth and Planetary Sciences, University of California, Berkeley, California, USA

Microbial chemosynthesis involves the use of reduced chemicals like sulfur to power carbon fixation and is pervasive throughout the deep sea. Chemosynthetic microbes drive key biogeochemical cycles that impact all life on earth. Viruses are the most abundant biological entities in the deep-sea and the primary cause of microbial mortality. Although viruses infecting phototrophs in the surface oceans are well studied, little is known about the impacts of viruses that infect lithotrophic primary producers. In this presentation, we will describe the ecological strategy of viruses that putatively infect the globally distributed 'SUP05' clade of sulfur-oxidizing gammaproteobacteria. We hypothesize that these viruses have acquired and retained bacterial metabolic genes to access abundant elemental sulfur in the environment. Metabolic genes in the viruses supplement sulfur oxidation metabolism in their hosts in order to support viral infection and replication. Our work implicates viruses as an agent of horizontal gene transfer of sulfur oxidation genes and an important component of the global biogeochemical cycle of sulfur.

L11

Characterisation of uncultured dimethylsulfide-degrading gammaproteobacteria from a coastal saltmarsh using stable isotope probing and single cell genomics

Hendrik Schäfer[1], Jennifer Pratscher[2], Hyun Soon Gweon[3], Eileen Muhs[1], Jonathan D. Todd[4], J. Colin Murrell[2], Andrew W. B. Johnston[4]

[1] School of Life Sciences, University of Warwick, Coventry, UK
[2] School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, UK
[3] Centre for Ecology and Hydrology, Wallingford, UK
[4] School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich, UK

Coastal saltmarshes are natural hotspots for organic sulfur transformations. Due to their high productivity and the presence of macroscopic and microscopic algae as well as macrophytes that have the capacity to produce dimethylsulfoniopropionate (DMSP), saltmarshes are environments where dimethylsulfide (DMS)-degrading microbial populations thrive in sediments and in association with plants, but little is known about the identity of DMS-degrading microorganisms in such environments. In this project we sought to identify the dominant populations and metabolic pathways in microbial catabolism of DMS in a saltmarsh at Stiffkey (Norfolk, UK). We combined stable isotope probing (SIP) with ^{13}C-labelled DMS, together with 454 high-throughput sequencing of microbial rRNA genes in heavy and light SIP fractions to identify DMS-degrading microorganisms in sediments and in association with *Spartina anglica*, a DMSP-producing halophyte. SIP experiments suggested that uncultivated gamma-proteobacteria from the *Piscirickettsiaceae* were actively involved in DMS catabolism. Metagenomic sequencing of ^{13}C-DNA from SIP-labelled benthic DMS-degrading populations and comparison to the ^{12}C-community DNA provided insights into particular functional genes enriched in the metagenome of the DMS-degrading bacteria. Single-cell genomics allowed us to characterise the phylogeny of these bacteria and enabled comparison of their genomes to *Methylophaga* spp., their closest cultivated DMS-degrading relatives. The analyses provide a first glimpse into the genomes of gammaproteobacterial DMS degraders which so far have resisted isolation but which may play key roles in DMS degradation in benthic and pelagic marine habitats.

L12

Ecogenomics of haloalkaliphilic sulphur bacteria

Gerard Muyzer

Microbial Systems Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands

Soda lakes are extreme environments with pH values between 9 and 11, and salinities up to saturation. However, despite these extreme conditions, soda lakes are highly productive and harbor diverse microbial communities. The sulfur cycle, driven by sulfur-oxidizing and sulfidogenic bacteria, is one of the most active element cycles in these habitats. Members of the genus Thioalkalivibrio have versatile metabolic capabilities, including sulfide oxidation, denitrification and thiocyanate utilization. We have isolated more than 70 strains, for which the Joint Genome Institute of the U.S. Department of Energy has sequenced the genomes. The availability of these sequence data will allow us to get insight into the diversity of these bacteria, their niche differentiation, and the molecular mechanisms by which they adapt to the extreme halo-alkaline conditions. For this we will use a systems biology approach, combining different 'omics' techniques with physiological experiments under well-defined conditions, and mathematical modeling. The results of these experiments are of paramount importance, both for a basic understanding of life under extreme conditions, as well as for the use of these bacteria in the sustainable removal of noxious sulfur compounds from waste streams. Here I will discuss the ecogenomics and application of haloalkaliphilic sulfur bacteria, and present the first results of comparative genomics.

Invited Lecture

L13

Thermospores of sulfate-reducing bacteria as biogeographic indicators

Casey Hubert

Department of Biological Sciences, University of Calgary, Canada

Sulfate-reducing bacteria (SRB) are well known inhabitants of the deep biosphere, yet subsurface habitats are leaky due to geological features such as hydrocarbon seeps, hydrothermal vents, discharging seamounts, and mud volcanoes. Geofluids may therefore passively transport subsurface dwelling microorganisms through and/or out of the subsurface. Consistent with this is the discovery of thermophilic SRB present as dormant endospores in surface marine sediments where in situ conditions are too cold to support their germination and growth. These "thermospores" are phylogenetically and physiologically diverse and many are closely related to *Clostridia* commonly found in deep anoxic habitats such as petroleum reservoirs or mid ocean ridges. The distribution of thermospores in marine sediments is spatially variable, both globally and regionally. In Arctic sediments near Svalbard, certain phylo- and phenotypes of sulfate-reducing *Desulfotomaculum* thermospores appear to be unique to certain fjords, while others are more widespread. In addition to surviving the cold temperatures in surface sediments, certain thermospores display remarkable heat resistance and remain viable after serial autoclaving and/or long-term exposure to temperatures above their growth limit. Several different thermospore taxa are typically detected in a single surface sediment location, suggesting multiple sources and dispersal vectors may contribute to this microbial diversity. Thermospores provide a tractable model for quantitative studies of microbial dispersal with the potential to highlight previously unconsidered dispersal mechanisms for marine microorganisms in general.

L14

The power of the rare: Sulfate reduction in an acidic peatland is driven by small networks of natively low abundant bacteria

Bela Hausmann[1], Klaus-Holger Knorr[2], Stephanie Malfatti[3], Susannah Tringe[3], Tijana Glavina del Rio[3], Mads Albertsen[4], Per H. Nielsen[4], Ulrich Stingl[5], Alexander Loy[1], Michael Pester[1, 6]

[1] Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna, Austria
[2] Hydrology Group, Institute of Landscape Ecology, University of Münster, Germany
[3] Joint Genome Institute, U.S. Department of Energy, Walnut Creek, California, USA
[4] Center for Microbial Communities, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Denmark
[5] Red Sea Research Center, King Abdullah University of Science and Technology, Saudi Arabia
[6] Chair for Microbial Ecology, Limnology and General Microbiology, Department of Biology, University of Konstanz, Germany

Peatlands are regarded primarily as methanogenic environments significantly contributing to global methane emissions. Little attention is given to the fact that dissimilatory sulfate reduction is maintained by a hidden sulfur cycle in these low-sulfate environments, with sulfate reduction rates being comparable to marine surface sediments. To deepen our understanding of sulfate reducers in peatlands, anoxic peat slurries were supplemented with typical degradation intermediates of organic matter at in situ concentrations and either stimulated with low amounts of externally supplied sulfate or incubated under endogenous conditions. Changes in the microbial community were monitored by 16S rRNA gene and cDNA amplicon sequencing and correlated to sulfate turnover. OTUs most abundant in the native community (Acidobacteria, Actinobacteria, Alphaproteobacteria, Planctomycetes) showed no significant response to sulfate stimulation. In contrast, small networks of natively low abundant bacteria strongly correlated with bulk sulfate turnover under lactate, propionate, and butyrate amendment. Among the responsive OTUs affiliated to recognized sulfate reducers, members of the genera *Desulfomonile* and *Desulfovibrio* (Deltaproteobacteria) responded specifically to one of these three substrates, while a *Desulfopila* OTU (Deltaproteobacteria) and a *Desulfosporosinus* OTU (Firmicutes) were always responsive, exhibiting a generalist lifestyle. Interestingly, the *Desulfosporosinus* OTU markedly increased its 16S rRNA and thus ribosome content but stayed at low abundance throughout the incubation period. This likely mirrors its ecological strategy in the natural peat soil. Sequencing of a metagenome enriched by DNA-stable isotope probing allowed almost complete reconstruction of the *Desulfosporosinus* population pan-genome and confirmed functional properties of this low abundant peatland sulfate reducer. The small networks of responsive OTUs always contained at least one member not affiliated to recognized sulfate reducers (e.g. Alphaproteobacteria) indicating possible metabolic interaction partners or novel sulfate reducers. In conclusion, our results show that selected low abundance microorganisms can have a profound effect on biogeochemical cycling and greenhouse gas production.

L15

Can cable bacteria actually oxidize sulfide? Insights from a genome

Lars Schreiber[1], Kasper Urup Kjeldsen[1], Jesper Jensen Bjerg[1], Andreas Bøggild[2], Jack van de Vossenberg[3], Lars Peter Nielsen[1, 2], Andreas Schramm[1, 2]

[1] *Department of Bioscience, Center for Geomicrobiology, Aarhus University, Denmark*
[2] *Department of Bioscience, Section for Microbiology, Aarhus University, Denmark*
[3] *Institute for Water Education, UNESCO-IHE, Delft, The Netherlands*

Cable bacteria are filamentous members of the family Desulfobulbaceae that can couple the reduction of oxygen at the surface of aquatic sediments to the oxidation of sulfide in anoxic layers centimeters below by an unknown electron conducting mechanism. The goal of the present study was to explore the metabolic and molecular basis for the unique lifestyle of the so far uncultured cable bacteria using single-filament genomics. Single cable bacterium filaments were isolated from Aarhus Bay sediment (Denmark) by micro-manipulation, their genomes amplified and sequenced. By combining the genomic information of two such filaments, an approx. 90% complete draft genome with a size of 3.7 Mbp was obtained. About 44% of the genome represented novel genes without homologs in other Desulfobulbaceae. Genes diagnostic for sulfide-oxidizing bacteria, e.g., encoding the Sox sulfur oxidation system, sulfide-quinone reductase or reverse-type dissimilatory sulfite reductase, were not found. Instead, the canonical dissimilatory sulfate reduction pathway was present, albeit lacking the typical membrane-associated electron transport proteins (DsrMKJOP), which in sulfate reducers couple the quinone pool with sulfite reduction. We propose that sulfide oxidation is performed via a reversal of the sulfate-reduction pathway, in which heterodisulfide reductases transfer electrons released by sulfide oxidation into the quinone pool. A proton translocating cytochrome *d* ubiquinol reductase may then couple the quinone pool to oxygen reduction. Besides by sulfide oxidation, the quinone pool may also be reduced by NADH via a proton and a sodium translocating NADH:ubiquinone oxidoreductase conferring the potential for both lithotrophic and organotrophic growth. Genes coding for the potentially electron-conducting, string-like structures found in the periplasm of cable bacteria were not identified. We did, however, identify homologs of multi-heme cytochromes involved in electron-transport in nanowires. These cytochromes along with pili could potentially be involved in the electron transport along the cable bacteria.

L16

Metagenomic study reveals first sulfate-reducing member of the Bacteroidetes-Chlorobi group

Vera Thiel[1], Jason Wood[2], David Ward[2], Donald Bryant[1, 3]

[1] Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA, 16802 USA
[2] Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, MT 59717, USA
[3] Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT 59717, USA

Dissimilatory sulfate reduction (DSR) plays major roles in prokaryotic energy transformation in oxygen-depleted environments as well as in sulfur and carbon cycling. Horizontal gene transfer is usually invoked to explain the polyphyletic distribution of DSR in Bacteria and Archaea. We identified a genomic bin defining a novel sulfate-reducing bacterium within a metagenome that was derived from the undermat layer of a phototrophic microbial mat from Mushroom Spring, Yellowstone National Park, WY, USA. The corresponding uncultured organism is predicted to be a motile, Gram-negative, anaerobic sulfate-reducing bacterium; it had previously been detected in the microbial mat by cloning and sequencing studies for *dsrAB*. Based on phylogenetic marker genes and BLAST hits, the closest characterized relative is *Ignavibacterium album*, a heterotrophic member of the Chlorobi. The metagenomic bin contains all genes necessary for DSR, and most of these genes are clustered. Phylogenetic analyses of DsrAB and AprAB, as well as the presence of *dsrT* and the absence of dsrEFH, support the assignment of this organism as a sulfate-reducing bacterium. The genes involved in DSR show significant similarity to sequences associated with uncultured sulfate-reducing bacteria in marine sediments. The metagenomic study of this hot spring microbial mat suggests the presence of the first sulfate-reducing member of the Bacteroidetes/Chlorobi group, organisms in which only sulfur-oxidizing bacteria were previously known. Comparative bioinformatics analysis supports the hypothesis that a genomic island for sulfate respiration may exist, and that horizontal transfer of this entire gene cluster can sometimes occur. As often observed for photosynthesis gene clusters, a few required genes were no longer a part of the hypothesized cluster, which suggests they may have been displaced after acquisition. Analysis of this unconventional *dsrAB*-containing member of Bacteroidetes/Chlorobi should provide new insights into the evolution of DSR and may possibly reveal a missing link between sulfate-reducing and sulfur-oxidizing prokaryotes.

L17

Ubiquitous Gammaproteobacteria dominate dark carbon fixation in coastal sediments

Stefan Dyksma, Bernhard Fuchs, Marc Mußmann

Max Planck Institute for Marine Microbiology, Germany

Nearly half of microbial dark carbon fixation in the oceans occurs in sediments, whereby oxidation of sulfur is considered the main carbon-fixing process. However, still very little is known about the identity and physiology of the key CO2-fixing and sulfide-detoxifying microorganisms in these sediments. We combined isotopic tracer experiments, the 16S rRNA approach and metatranscriptomics to identify potential key chemolithoautotrophic populations in nine coastal surface sediments in Australia and Europe. Using a novel methodological approach we were able to quantify carbon fixation by specific populations. Sediment incubations with ^{14}C bicarbonate, subsequent flow-sorting of FISH-identified cells and liquid scintillography of the sorted cell fractions showed that Gammaproteobacteria accounted for 70-86% of carbon fixed by the total bacterial community. In accordance, 16S rRNA pyrotag sequencing suggested relatives of gammaproteobacterial, sulfur-oxidizing symbionts and the Acidiferrobacter group as the main chemolithoautotrophic populations at all sites. Together with the physiologically yet-unknown JTB255 group these core groups accounted for 36-67% of gammaproteobacterial sequences and for 62% of gammaproteobacterial carbon fixation. In support, environmental transcripts of sulfur oxidation and carbon fixation genes mainly affiliated with those of uncultured sulfur-oxidizing Gammaproteobacteria. Unexpectedly, gammaproteobacterial genes encoding Ni, Fe uptake hydrogenases recruited many transcripts. The co-localization and expression of key genes of sulfur- and hydrogen oxidation in a metagenomic fragment and in a single cell genome strongly suggest that sulfur-oxidizing Gammaproteobacteria in coastal sediments can cope with the fluctuating availability of energy sources. In accordance, sediment incubation experiments demonstrated a sulfate-independent oxidation of hydrogen. The role of hydrogen oxidation in dark carbon fixation remains unclear, but this finding provides a novel perspective on benthic primary production that has been ignored before. Our study indicates a central function of these ubiquitous, uncultured Gammaproteobacteria for sulfide detoxification and as a yet-overlooked carbon sink in marine sediments.

L18

Metagenomes from the terrestrial deep biosphere reveal sulfur oxidation and reduction pathways

Xiaofen Wu[1], Karin Holmfeldt[1], Valerie Hubalek[3], Mats Åström[2], Stefan Bertilsson[3], Mark Dopson[1]

[1] Centre for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, Kalmar, Sweden
[2] Department of Biology and Environmental Science, Linnaeus University, Kalmar, Sweden
[3] Department of Ecology and Genetics, Limnology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Despite scarce carbon and energy sources, microorganisms in the deep terrestrial subsurface are estimated to constitute approximately 20% of the earth's biomass. However, due to the difficulties of accessing samples, details of the diversity and metabolism of these microorganisms remain largely unexplored. We analyzed the microbiomes of three dominant deep biosphere water types ("modern marine", "old saline", and an "undefined mix") at the Äspö Hard Rock Laboratory, Sweden. The metagenome data has been assigned to twenty-four genome bins constituting near complete genomes of the dominating microorganisms from the three water types. Most of the bins were classified as similar to uncultured and uncharacterized bacteria while four belong to unclassified archaea. Metagenomic bins related to known microbial species include a *Thiobacillus denitrificans*-like species identified from the old saline water that exhibits *soxA* from the Sox enzyme complex and *asrAB* coding for subunits of a reversible anaerobic sulfite reductase. Co-occurrence of these genes may suggest coupling of inorganic sulfur oxidation to dissimilatory nitrate reduction. In addition, a *Desulfarculus baarsii*-like population was found in the old saline water that contains genes coding for the reductive acetyl coenzyme A pathway that oxidizes carbon sources such as formate and oxalate potentially coupled to sulfate as the terminal electron acceptor. These metagenomes will for the first time allow detailed investigation of the molecular mechanisms underpinning microbial sulfur transformations in the deep biosphere.

L19

Tight symbiotic interactions in the pelagic sulfur cycle – the case of phototrophic consortia

Petra Henke[1], Johannes Müller[1], Gerhard Wanner[2], Shawn McGlynn[3], Victoria Orphan[3], Jörg Overmann[1]

[1] Leibniz Institute DSMZ - German Collection of Mikroorganisms and Cell Cultures, Germany
[2] Ludwig Maximilian University of Munich, Germany
[3] California Institute of Technology, USA

Green sulfur bacteria are obligate photolithoautotrophs inhabiting a very narrow ecological niche characterized by the simultaneous presence of light and sulfide. Several green sulfur bacteria have gained the advantage of mobility by entering into symbiosis with motile Betaproteobacteria forming multicellular associations termed phototrophic consortia. In the first cultured model system "Chlorochromatium aggregatum" the chemoheterotrophic central bacterium is surrounded in a highly ordered fashion by 12-20 green sulfur bacteria epibionts. Transcriptomics analyses of intact consortia and pure epibiont cultures revealed that of 328 differently expressed genes, 25 genes are involved in amino acid pathways (1). Metabolic coupling appears to involve amino acids and was studied by tracking the flux of isotopically-labeled CO_2 through the two partner organisms using NanoSIMS analysis and magnetic capture. The epibiont genome also contains a limited number of unique putative symbiosis genes (1,2). Three of the putative symbiotic genes (Cag_1919, Cag_0614, Cag_0616) were studied in more detail and their intracellular localizations determined. All three postulated symbiotic genes are transported across the cell envelope of the epibiont into the central bacterium. Cag_1919 contains a RTX domain which is typically found in Gram-negative pathogenic bacteria. Cag_0614 and Cag_0616 represent the largest open reading frames in the prokaryotic world known to date. Interestingly, Cag_0616 is predicted to be transcribed together with a polysulfide reductase and Cag_0614 contains signature sequences for the α- and β-ATP synthase subunits and might constitute a monitoring system for motility of the consortium. The results of our physiological, localization and bioinformatic studies yield novel hypotheses with respect to tight symbiotic interactions within the pelagic sulfur cycle.

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L20

Single-cell investigations into the metabolism of stored sulfur in living bacteria

Jasmine Berg, et al.

Max Planck Institute for Marine Microbiology, 28359 Bremen, Germany

Zero-valent sulfur is a key intermediate in the microbial oxidation of sulfide to sulfate. Many sulfide-oxidizing bacteria therefore produce and store large amounts of sulfur intracellularly. How this stored sulfur is utilized remains a subject of discussion as the most stable from of S^0 under standard biological conditions – orthorhombic α-sulfur – is most likely not accessible to bacterial enzymes. In this study (Berg et al. 2014), Raman spectroscopy was employed as an ideal non-destructive technique to investigate potentially redox- and pH-sensitive sulfur species in single cells of living bacteria. We compared the chemical nature of sulfur in four different strains of *Beggiatoa* under various ecological and physiological conditions. Results showed that in microaerobic cultures at circumneutral pH, stored intracellular sulfur consisted of S_8 rings and inorganic polysulfides (S_n^{2-}). Linear sulfur chains were detected during both the oxidation and reduction of stored sulfur suggesting that S_n^{2-} species comprise a pool of activated sulfur utilized by bacteria. The formation of S_n^{2-} results from the cleavage of sulfur rings, either biologically by membrane-bound thiol groups and glutathione or chemically by the strong nucleophile HS^-. It is likely that Beggiatoa in the environment utilize both of these mechanisms to generate S_n^{2-} intermediates as they migrate vertically between oxic and sulfidic sediment zones. With Raman spectroscopy it was also possible to further follow the fate of sulfur during its oxidation to sulfate. Unexpectedly high concentrations (up to ~2 M) of internal sulfate were detected in *Beggiatoa* sp. Although the reason for the intracellular accumulation of sulfate remains unknown we could show for the first time that *Beggiatoa* contains sulfate in concentrations 100-1,000-fold higher than in the environment.

L21

Microbial carbon metabolism associated with electrogenic sulphur oxidation in coastal sediments

Diana Vasquez Cardenas[1], Jack van de Vossenberg[1], Lubos Polerecky[2], Sairah Malkin[3], Regina Schauer[4], Silvia Hidalgo-Martinez[1], Veronique Confurius[1], Jack Middelburg[2], Filip Meysman[1, 3], Henricus Boschker[1]

[1] Royal Netherlands Institute for Sea Research, Yerseke, The Netherlands
[2] Department of Earth Sciences, Utrecht University, Utrecht, the Netherlands
[3] Department of Environmental, Analytical and Geo-chemistry, Vrije Universiteit Brussel, Brussels, Belgium
[4] Department of Bioscience, Aarhus University, Aarhus, Denmark

Recently, a novel electrogenic type of sulphur oxidation was documented in marine sediments, whereby filamentous cable bacteria (Desulfobulbaceae) mediate electron transport over cm-scale distances. These cable bacteria are capable of developing an extensive network within days, which implies a highly efficient carbon acquisition strategy. Presently the carbon metabolism of cable bacteria is unknown, so we adopted a multi-disciplinary approach to study the carbon substrate utilization of both cable bacteria and associated microbial community in sediment incubations. Fluorescence in situ hybridization showed rapid downward growth of cable bacteria, concomitant with high rates of electrogenic sulphur oxidation, as quantified by microelectrode profiling. We studied heterotrophy and autotrophy by following ^{13}C-propionate and -bicarbonate incorporation into bacterial fatty acids. This biomarker analysis showed that propionate uptake was limited to fatty acid signatures typical for the genus Desulfobulbus. NanoSIMS analysis confirmed heterotrophic rather than autotrophic growth of cable bacteria. Still high bicarbonate uptake was observed in concert with the development of cable bacteria. Clone libraries of 16S cDNA showed numerous sequences associated to chemoautotrophic sulphur-oxidizing Epsilon- and Gammaproteobacteria while ^{13}C-bicarbonate biomarker labelling suggested that these sulphur-oxidizing bacteria were active far below the oxygen penetration. A targeted manipulation experiment demonstrated that chemoautotrophic carbon fixation was tightly linked to the heterotrophic activity of the cable bacteria down to centimetres depth. Overall, results suggest that electrogenic sulphur oxidation is performed by a microbial consortium, consisting of chemo-organotrophic cable bacteria and chemo-lithoautotrophic Epsilon- and Gammaproteobacteria. The metabolic linkage between these two groups is presently unknown and needs further study.

L22

Metagenome and mRNA expression analysis of the bacterial partner of an AOM-mediating microbial consortium

Jon Graf, Jana Milucka, Timothy Ferdelman, Marcel Kuypers

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

Sulfate-coupled anaerobic oxidation of methane (AOM) is a major sink of methane in the ocean and plays an important role in sedimentary biogeochemical cycling of carbon and sulfur [1]. The Deltaproteobacteria belonging to the *Desulfosarcina*/*Desulfococcus* (DSS) clade associated with the methanotrophic archaea (ANME) are capable of polysulfide disproportionation and couple the carbon and sulfur cycles during AOM [2]. Biochemical studies of elemental sulfur and thiosulfate disproportionation in *Desulfocapsa sulfoexigens* have shown that enzymes involved in canonical sulfate reduction (sulfate adenylyl transferase, adenylylsulfate reductase, sulfite reductase) are also mediating thiosulfate and likely elemental sulfur disproportionation [3]. Using differential coverage binning we have isolated a draft genome of the AOM bacterial partner from a metagenome of a highly enriched AOM culture originating from sediments of the Mediterranean mud volcano Isis. 228 contigs constituted the 3.7 Mb draft genome containing a full length 16S ribosomal DNA clustering within the SEEP-SRB1 group of DSS. Metagenome binning validation using essential single copy gene (ESCG) analysis [4] indicated that the draft genome was almost complete and contamination-free (101 of 104 unique ESCG, 3 duplicates). Within the draft genome we found all key genes for the canonical sulfate reduction pathway (Sat, AprAB, DsrAB) as well as genes encoding for putative accessory proteins for sulfate reduction (e.g. DsrC, inorganic pyrophosphatase). mRNA expression analysis showed that these genes were among the 10 highest expressed genes and thus are likely involved in sulfur metabolism of DSS. Furthermore we found in immediate proximity to Sat and Apr genes a well expressed putative operon comprised of four molybdopterin oxidoreductases, two of which show high similarity to polysulfide reductase (NrfD-type family).

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Invited Lecture

L23

What is the physiological product of the DsrAB dissimilatory sulfite reductase?

Inês A. C. Pereira[1], André Santos[1], Sofia S. Venceslau[1], Fabian grein[1], William D. Leavit[2], David T. Johnston[2], Christiane Dahl[3]

[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
[2] Department of Earth & Planetary Science, Harvard University, Cambridge, MA, USA
[3] Institut for Microbiology and Biotechnology, University of Bonn, Germany

Many questions remain about how energy is conserved in sulfur-metabolizing organisms. A key reaction in microbial sulfur metabolism is the reduction of sulfite by the dissimilatory sulfite reductase, DsrAB. This enzyme is present in sulfate, thiosulfate and sulfite reducing organisms, and also in sulfur-oxidizers. The mechanism of sulfite reduction by DsrAB has long been the subject of controversy due to the in vitro formation of thiosulfate and trithionate, in contrast to the closely-related assimilatory enzyme that produces only sulfide. Recent studies have identified the small protein DsrC [1] and the DsrMKJOP membrane complex as physiological partners of DsrAB [2]. In particular, a crystal structure of DsrAB in complex with DsrC suggested the involvement of the latter protein in sulfite reduction and led to the proposal of a new mechanism for this reaction [3]. I will present recent in vivo and in vitro studies that reveal the function of DsrC in sulfite reduction, identifying the mechanism and physiological product of this reaction. These results implicate the respiratory membrane complex DsrMKJOP in the process, providing a direct link to energy conservation.

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L24

New insights into the energy metabolism of *Desulfovibrio vulgaris*: The role of FlxABCD-HdrABC, a novel NADH dehydrogenase-heterodisulfide reductase

Sofia Venceslau[1], Ana Raquel Ramos[1], Fabian Grein[1], Irene Raccagni[1], Gonçalo Oliveira[1], Kimberly Keller[2], Judy Wall[2], Inês Pereira[1]

[1] *Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal*
[2] *University of Missouri, Biochemistry Department, Columbia, USA*

Flavin-based electron bifurcation (FBEB) has been recently recognized as an important process for the energy metabolism of anaerobic organisms [1]. Here, we report on a new family of proteins, the Flavin oxidoreductase (FlxABCD), which is a new NADH dehydrogenase that, together with a heterodisulfide reductase (HdrABC), seems to be involved in FBEB [2]. The *flxABCD* genes are usually found next to *hdrABC* genes [3], and this gene cluster is found in a large number of anaerobes, suggesting a general and important role in their bioenergetics. In the case of the sulfate reducing organism *Desulfovibrio vulgaris* Hildenborough the *hdr-flx* genes are part of the same transcriptional unit. The levels of transcripts and proteins of the *hdr-flx* gene cluster were increased in growth with ethanol/sulfate and to a less extent in pyruvate fermentation. Two mutant strains were generated, one lacking expression of the *hdr-flx* gene cluster and a Δ*flxA* mutant. Both mutants were impaired in growth with ethanol/sulfate, whereas growth was restored in a *flxA*-complemented strain. Furthermore, the wild-type and complemented strain produce ethanol as a product of fermentation, which is not observed with both mutants. Our results show that in *D. vulgaris* the FlxABCD-HdrABC proteins are essential for NADH oxidation during growth on ethanol, probably involving a FBEB mechanism that couples reduction of ferredoxin and DsrC, whereas in fermentation they operate in reverse, reducing NAD^+ for ethanol production. This provides the first link between NADH and sulfate (sulfite) reduction through the DsrC protein [2,4].

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L25

Crucial role of DsrC in dissimilatory sulfite reduction

André A. Santos, Sofia S. Venceslau, Fabian Grein, Inês A. C. Pereira

Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

The difference between assimilatory and dissimilatory sulfite reduction is an important question that remains to be answered. Both assimilatory and dissimilatory sulfite reductases contain the same unique catalytic site: a siroheme-[4Fe4S] coupled cofactor. It is also known that the assimilatory sulfite reductase reduces sulfite directly to sulfide. On the other hand several in vitro studies revealed that the dissimilatory sulfite reductase (DsrAB) reduces sulfite to a mixture of trithionate, thiosulfate and sulfide. In 2008 Oliveira and coworkers proposed a new mechanism where DsrAB cooperates with another protein (DsrC) to reduce sulfite to sulfide. The present work reveals the relationship between DsrAB and DsrC. DsrC increases the activity of DsrAB in sulfite reduction without formation of sub-products. Our results show that DsrC is directly involved in dissimilatory sulfite reduction and allow the identification of the mechanism and physiological product of this essential reaction.

L26

Chemolithoheterotrophy – new insights into an often forgotten yet widespread metabolic trait

Rich Boden, Lee Hutt

University of Plymouth, UK

Oxidation of sulfur anions by heterotrophic Bacteria has been recognised for over 100 years. This can be divided into the so-called 'gratuitous oxidation', occuring with no apparent increase in yield or benefit to the organism and 'fortuitous oxidation' in which increases in yield and/or specific growth rate may occur due to additional ATP/[H] produced in S-oxidation – chemolithoheterotrophy. This is not to be confused with mixotrophic metabolic modes in which sulfur oxidation supports chemolithoautotrophic growth occurring simultaneously with heterotrophic growth, as observed in *Paracoccus* spp., for example.

We have revisited sulfur oxidation in a number of strains historically dubbed "Thiobacillus trautweinii" - viz. *Pseudomonas* sp. T (Trautwein, 1921) and *Achromobacter* sp. B (Starkey, 1934). In addition to confirming the nature of the sulfur oxidation pathways and enzymes involved as well as understanding the bioenergetic benefits to the organism we have understood the triggers behind fortuitous vs gratuitous oxidation. We have demonstrated that chemolithoheterotrophy is not triggered specifically by carbon-limited growth as has previously been proposed as identical increases in yield can be observed in chemostats limited by carbon, oxygen or by phosphate when media are supplemented with thiosulfate rather than in growth on sugars or organic acids alone.

Thiosulfate dehydrogenase (EC. 1.8.2.2) activity was found in all strains examined and expression was induced by the presence of thiosulfate, however, this alone was not sufficient to produce an increase in yield. In batch cultures in which cells are not limited by intracellular ATP levels, no increase in yield occurs but in the chemostat in which cells are limited by ATP, an intracellular “ATP cap” acts as the trigger – when ATP levels in the cell fall below this ‘cap’, additional ATP from sulfur oxidation results in a yield increase, but when ATP in cells is above it, no benefit occurs.

Invited Lecture

L27

An integrated view on sulfur oxidation in purple sulfur bacteria

Christiane Dahl

Institut für Mikrobiologie & Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany

Among the sulfur-oxidizing anoxygenic phototrophic bacteria, the Gamma-proteobacterium *Allochromatium vinosum* has been developed into a model organism not only for biochemical and structural analysis of enzymes involved in oxidative sulfur metabolism but also for a systems biology approach including comparative genome analysis, genome-wide transcriptional profiling, differential expression proteomics and metabolomic profiling (1, 2). Thus, a rather comprehensive and coherent picture of bioenergetic processes and sulfur oxidation via the Dsr (dissimilatory sulfite reductase) pathway can now be provided. Classical reverse genetics and in vitro analyses of sulfur transfer reactions via tandem mass spectrometry further aided the detection of new genes/proteins participating in oxidative sulfur metabolism. The identification of the cytoplasmically oriented sulfite-oxidizing iron-sulfur molybdoprotein SoeABC as a major player in the oxidation of sulfite to sulfate and the detection of extensive sulfur trafficking networks involving rhodanese, TusA, DsrE-like proteins, DsrEFH and DsrC in the cytoplasm *A. vinosum* (3) serve as examples. An *rhd-tusA-dsrE2* or at least a *tusA-dsrE2* arrangement also occurs in many photo- and chemotrophic sulfur oxidizers that do not contain the Dsr pathway. Those sulfur oxidizers include members of the genera *Thiorhodospira*, *Ectothiorhodospira*, *Thioalkalivibrio* and *Acidithiobacillus*. Here, the *tusA-dsrE2* genes are linked with genes encoding a possible heterodisulfide reductase complex, (a) liponamide-binding protein(s) and proteins involved in biosynthesis of the latter. These findings again incite discussion about previous suggestions of a new sulfur oxidation pathway involving an HdrC1B1AHypHdrC2B2 complex (4).

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Invited Lecture

L28

Sulfur and beyond: Energy sources for sulfur-oxidizing symbionts

Jillian M. Petersen

Symbiosis Department, Max Planck Institute for Marine Microbiology, Bremen, Germany

Chemosynthetic symbioses between sulfur-oxidizing bacteria and marine invertebrates were first discovered in 1977 at deep-sea hydrothermal vents but are now known to occur in a wide range of habitats including coral reef sediments, seagrass beds, cold seeps and sunken whale carcasses. On the host side, these associations have evolved multiple times in convergent evolution in at least 9 animal groups such as flatworms, annelids, nematodes, mussels, clams, and snails. Similarly, chemosynthetic symbionts have evolved – and are continuing to evolve – from numerous bacterial lineages. Many are so closely related to free-living chemosynthetic bacteria that at the 16S rRNA level, they can be considered to belong to the same genus or even the same species (Dubilier et al. 2008).

For the first thirty years after their discovery, sulfur-oxidizing symbionts were assumed to use reduced sulfur compounds as their sole energy sources. Only a few studies have examined the genomic potential for other energy sources and even fewer have investigated which energy sources are used in situ. We recently discovered that chemosynthetic symbionts use a surprisingly wide range of energy and carbon sources that includes hydrogen, carbon monoxide, and organic carbon compounds (Petersen et al., 2011 Nature, Kleiner et al. 2012 PNAS, Unpublished results). In my talk, I will present an overview of my research in this area and discuss if the metabolic versatility of both symbiotic and free-living sulfur-oxidizing bacteria may be much greater than previously recognized. I will also discuss the role of horizontal gene transfer as the mechanism driving this metabolic versatility.

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L29

Oxidation of thiosulfate and sulfite by the hyperthermophilic bacterium *Aquifex aeolicus*

Souhéla Boughanemi, David Ranava, Marie-Thérèse Giudici-Orticoni, Marianne Guiral

Bioenergetics and Protein Engineering Laboratory, CNRS-Aix-Marseille University, Mediterranean Institute of Microbiology, 31 chemin Joseph Aiguier, 13402 Marseille cedex 20, France

Aquifex aeolicus is a hyperthermophilic, chemolithoautotrophic and microaerophilic bacterium that uses molecular hydrogen or inorganic sulfur compounds as electron donor to grow. On the basis of genome analysis and biochemical studies, we have proposed a general model of its sulfur metabolism. A significant effort has been made to characterize the enzymes of this metabolism, but much remains to be done to better understand the oxidation of sulfur compounds. We have shown using an Oxygraph that oxygen is consumed by entire cells or membrane fraction, with thiosulfate or sulfite as electron donor and developed a protocol to visualize both the thiosulfate- and sulfite-oxidase activity directly in native gel. At least one protein can oxidize these sulfur compounds in vitro with an artificial electron acceptor. This protocol allowed us to (i) localize the thiosulfate-oxidase and sulfite-oxidase activity in the membrane fraction, and (ii) demonstrate that the two activities are localized together in the gel, suggesting that they arise from the same molecular entity, a complex of about 500 kDa. With the aim of characterizing this complex, we have started to purify it from the membrane of A. aeolicus. Mass spectrometry analysis of a partially purified fraction indicated that it contains a heterodisulfide reductase (HdrABC) and additional proteins encoded by the hdr operon as well as a molybdenum-dependant three-subunit enzyme annotated as DMSO reductase. This last, which we had already characterized as a sulfur reductase, might also catalyze sulfur compounds oxidation and could be, as proposed in the sulfur bacterium *Allochromatium vinosum*, a cytoplasmic sulfite-oxidizing complex that was missing from the sulfur metabolism of *A. aeolicus*. We are currently purifying this molybdenum oxidase as well as the Hdr, which seems to be an essential enzyme in bacterial energy sulfur metabolism, to shed light on their metabolic role.

L30

Organosulfur compound metabolism in the human pathogen *Haemophilus influenzae*

Dk Seti Maimonah Othman[1], Noor Marian Muda[1], Rabeb Dhouib[1], Horst J Schirra[1, 2], Alastair G McEwan[1], Ulrike Kappler[1]

[1] School of Chemistry and Molecular Biosciences, The University of Queensland, Australia
[2] Centre for Advanced Imaging, The University of Queensland, Australia

Non-typeable *Haemophilus influenzae* (NTHi) is a host-adapted pathogen that causes a variety of acute and chronic infections including otitis media, non-CF bronchiectasis and chronic obstructive pulmonary disease (COPD). NtHi generates energy via a 'respiration assisted fermentation' that relies on a mixed acid type fermentation linked to a versatile respiratory chain used for redox balancing. This respiratory chain contains two putative sulfoxide converting molybdenum enzymes, a DmsA-type dimethylsulfoxide (DMSO) reductase and a TorZ-like enzyme. Both enzymes were highly expressed under mostly anaerobic conditions, which is in keeping with established roles and expression patterns for such enzymes in e.g. *E. coli*. However, the main substrates for either of these enzymes, DMSO and trimethylaminoxide (TMAO), are largely absent from the human body, calling into question the physiological significance of these enzymes is in H. influenzae.

Our data show that both enzymes are conserved in Pasteurellaceae, and form separate clades within their respective enzyme families, indicating important roles in pathogen physiology. In order to elucidate the properties and physiological roles of these enzymes, we have created and studied *dmsA* & *torZ* mutant strains of *H. influenzae*. The Δ*dmsA* strain showed defects in biofilm formation and colonization of epithelial cells, while the Δ*torZ* strain showed minor changes in tissue cell colonization. Our characterization of the purified TorZ enzyme showed low affinities to DMSO and TMAO, but high affinity for methionine sulfoxide as a substrate, further corroborating the fact that this group of enzymes fulfils a novel function in bacterial physiology. Gene expression patterns varied for the two sulfoxide reductases varied in different strains of *H. influenzae*, which may be indicative of niche specific adaptation. Based on our results we propose that DmsA & TorZ may act on sulfoxides produced from e.g. sulfur containing amino acids during interaction of NTHi with the immune system.

Invited Lecture

L31

The biosynthesis of the molybdenum cofactor and its relation to sulfur metabolism

Silke Leimkuehler

University of Potsdam, Germany

Molybdenum is the only second row transition metal essential for biological systems, which is biologically available as molybdate ion. In eukarya, bacteria and archaea, molybdenum is bound to a tricyclic pyranopterin, thereby forming the molybdenum cofactor (Moco). To date more than 50 Moco-containing enzymes have been purified and biochemically or structurally characterized. The physiological role of molybdenum in these enzymes is fundamental to organisms, since the reactions include the catalysis of key steps in carbon, nitrogen and sulfur metabolism. The catalyzed reactions are in most cases oxo-transfer reactions or the hydroxylation of carbon centers. The biosynthesis of Moco has been intensively studied, in addition to its insertion into molybdoenzymes. In particular, a link between the biosynthesis and maturation of molybdoenzymes and the biosynthesis and distribution of FeS clusters has been identified in the last years. Here, both pathways are directly linked by the sulfur mobilizing enzyme in the cell: The sulfurtransferase for the dithiolene group in Moco is common also for the synthesis of FeS clusters, thiamin and thiolated tRNAs. Here, the main focus is on the biosynthesis of the molybdenum cofactor in bacteria, its modification and insertion into molybdoenzymes, with an emphasis to its link to FeS cluster biosynthesis and sulfur transfer in the cell.

L32

Comparative metabolic studies of the halo-alkaliphilic chemolithoautotrophic sulfur-oxidizing bacterium *Thioalkalivibrio thiocyanoxidans* ARh2

Tom Berben[1], Dimitry Sorokin[2, 3], Gerard Muyzer[1]

[1] *Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands*
[2] *Winogradsky Institute of Microbiology, RAS, Moscow, Russia*
[3] *Department of Biotechnology, Delft University of Technology, Delft, The Netherlands*

Soda lakes are characterized by their extremely high pH and moderate to high salinity. Carbonate is the dominant anion, leading to a uniquely stable sodium carbonate/bicarbonate buffer system which has a maximum buffering capacity at pH 9.5-10. Despite these extreme conditions, soda lakes harbor a rich biodiversity that drives active biogeochemical cycles, of which the sulfur cycle is one of the most active. One of the dominant genera of sulfur oxidizers is *Thioalkalivibrio*, a group of high salt-tolerant, alkaliphilic, chemolithoautotrophic Gammaproteobacteria. They are capable of oxidizing a variety of inorganic sulfur compounds, such as sulfide, thiosulfate, elemental sulfur and tetrathionate. Some strains also have the ability to use the C-1 sulfur compound thiocyanate (NCS^-) as electron-donor, sulfur and nitrogen source. We have performed chemostat cultivations of *Thioalkalivibrio thiocyanoxidans* ARh2 grown on either thiosulfate or thiocyanate followed by transcriptomics analysis aiming to uncover difference in enzymatic profiles expressed with two different substrates. Furthermore, comparative analysis on a large set of *Thioalkalivibrio* genomes sequenced at the DOE's Joint Genome Institute has raised additional questions regarding the physiology and biochemistry of sulfur oxidation in this group. In the absence of the sulfane dehydrogenase SoxCD, the dissimilatory sulfite reductase (DSR) pathway running in the reverse direction is the only known alternative to oxidize zero-valent sulfur to sulfite, but comparative genomics has shown that many sequenced genomes of *Thioalkalivibrio* lack the DSR cluster as well as SoxCD. A systems biology approach using transcriptomics was used to investigate the differential expression of genes involved in the sulfur oxidation in *T. thiocyanoxidans* ARh2. Closing the gaps in our knowledge of the sulfur cycle is essential for creating a complete picture of the microbial ecology in soda lakes.

L33

Ecology and ecogenomics of uncultured sulfate-reducing bacteria ubiquitous and abundant in marine sediments

Marc Mussmann, Stefan Dyksma, David Probandt, David Virant, Kin Ovanesov, Sten Littmann

Max Planck Institute for Marine Microbiology, Germany

Sulfate reduction is the key electron accepting process that drives anaerobic carbon mineralization in marine coastal sediments. Despite this, we still know little about the ecology and ecophysiology of the sulfate-reducing bacteria (SRB) responsible. We used 16S rRNA gene pyrotags and fluorescence in situ hybridization (FISH) to investigate the identity and abundance of SRB in 9 coastal sediments in Europe and Australia. The uncultured Sva0081-group, which includes the sulfate-reducing endosymbionts of the gutless oligochaete *Olavius* sp., and was first described in clone libraries from Svalbard sediments, was a ubiquitous and highly abundant member of SRB communities in diverse habitats from the deep sea to intertidal sediments. Single-cell genomes and metatranscriptomes revealed that they use diverse energy sources including organic acids, aromatic compounds and hydrogen. Expression of high-affinity transporters for carbohydrates, dicarboxylates, polyamines and amino acids/peptides shows that a diverse range of substrates is used in situ. The broad array of defense mechanisms against oxidative stress that are encoded in Sva0081-SRB genomes could explain their remarkable ability to thrive in fluctuating environments such as tidal sediments. Genome comparisons with other SRB revealed potential physiological properties unique to Sva0081-SRB compared to other known SRB. For instance, we identified a highly expressed high-affinity ABC phosphate transporter that had not been previously found in SRB. Quantification of phosphorus levels in single Sva0081-cells via SEM-EDS suggested that cells from the uppermost tidal sediment layer (0-2.5 mm) accumulate significantly more phosphorus/phosphate than cells from deeper layers. The highly versatile metabolism of the Sva0081-SRB may provide them with a distinct competitive advantage, and could explain their remarkable success in diverse marine habitats.

Invited Lecture

L34

Applications of the biological sulfur and selenium cycles in environmental biotechnology

Piet Lens

UNESCO-IHE Institute for Water Education, Westvest 7, 2611 AX Delft, The Netherlands

Invited Lecture

L35

Sulfur-metabolizing microorganisms in oil degradation and corrosion

Ian Head[1], Ana Suarez-Suarez[1], Luiza Andrade[1, 2], Angela Sherry[1], Sven Lahme[1], Carolyn Aitken[1], Martin Jones[1], Neil Gray[1], Casey Hubert[1, 3]

[1] School of Civil Engineering and Geosciences, Newcastle University, UK
[2] Instituto Nacional de Tecnologia –INT, Av. Venezuela, Rio de Janeiro, Brasil
[3] Department of Biological Sciences and Energy Bioengineering and Geomicrobiology Group, University of Calgary, Canada

The significance of sulfur-metabolizing microorganism for petroleum and petroleum systems was first recognized as long ago as the 1920s when Bastin first isolated sulfate-reducing bacteria from petroleum reservoirs in Illinois. Since those seminal studies, the reach of microbial sulfur metabolism in petroleum microbiology has ranged from fundamental new discoveries in microbial hydrocarbon metabolism to the recognition of the role of sulfur metabolizing microorganisms in economically significant processes such as petroleum reservoir souring, corrosion and oil biodegradation. Exploration of the microbial metabolism of individual hydrocarbon compounds by sulfate-reducing bacteria has transformed our appreciation of what is biochemically possible in anoxic systems with the discovery of novel mechanisms for activation of stable hydrocarbons. However relatively little work has been conducted on the microbial metabolism of crude oil, one of the most complex natural substances on Earth, containing tens of thousands of different compounds resolvable with state-of-the-art high resolution mass-spectrometry techniques. The role of sulfate-reducing microorganisms in transformations of a range of saturated and aromatic hydrocarbons in crude oil will be explored both from the view-point of their importance in attenuation of oil pollution in anoxic sediments but also in relation to their operational significance to the oil industry and understanding fundamental questions about the deep biosphere. The relationship between petroleum and sulfur bacteria also encompasses sulfide-oxidizing bacteria which have been implicated as agents in nitrate-mediated control of reservoir souring. However these organisms may have a dual personality leading to unintended consequences in the form of microbial influenced corrosion. The many facets of the interaction of the sulfur cycle with petroleum systems will be explored in this presentation.

L36

Development and application of acidophilic sulfidogenic bioreactors for combined pH amelioration, sulfate removal and selective recovery of metals from acidic waste waters

Ana Laura Santos[1], Roseanne Holanda[1], Sabrina Hedrich[2], Ivan Ňancucheo[3], Carmen Falagán[1], Barry Grail[1], Barrie Johnson[1]

[1] College of Natural Sciences, Bangor University, Bangor LL57 2UW, UK
[2] Federal Institute for Geosciences and Natural Resources (BGR), Stilleweg 2, 30655 Hanover, Germany
[3] Instituto Tecnológico Vale, Belém, Brazil

Acidic, sulfate-enriched waste waters can be generated by different industrial processes. Mining of metals and coals is notorious in this context, often producing waste streams that also contain elevated concentrations of various transition metals and aluminium. Dissimilatory biosulfidogenesis (the production of hydrogen sulfide by microbial reduction of sulfate and other more oxidized forms of sulfur in anoxic environments) has great potential for ameliorating such waste waters. Biosulfidogenesis is an acid-consuming process (at pH <7), lowers concentrations of soluble sulfate, and the hydrogen sulfide generated can react with chalcophilic metals present to produce insoluble sulfide phases. We have developed continuous flow biofilm sulfidogenic reactors which, in contrast to similar systems, are populated by consortia of acidophilic and acid-tolerant sulfate-reducing bacteria, and operate effectively at set pH values between pH 2.5 and 5. The indigenous communities respond to changes in operating pH values by, for example, more acidophilic species becoming more dominant at lower pH values, and vice-versa. We have used the reactors (2 L, working volume) to increase the pH of acidic, metal-rich waste waters, lower their concentrations of sulfate, and to remove metals selectively, thereby facilitating their recovery and recycling. The modular units are highly versatile, operate with minimal control, and can be readily configured to remediate acidic waters of widely different chemical compositions where the primary objectives (pH amelioration, sulfate removal or metal recovery) may vary. Examples will be presented where the acidophilic sulfidogenic bioreactors have been used to remove sulfate from acidic groundwater (Germany) and mine process water (Chile), and to selectively recover transition metals (copper, zinc etc.) from metal mine drainage waters in Sweden, Brazil and Wales.

L37

Oxidation of inorganic sulfur compounds in metal sulfide processing wastewaters generates an electrical current in microbial fuel cells

Gaofeng Ni, Mark Dopson

Linnaeus University, Sweden

Acidophilic microorganisms optimally grow at low pH that is often generated by the oxidation of inorganic sulfur compounds to sulfuric acid. An abundant source of inorganic sulfur compounds, such as tetrathionate and thiosulfate, is wastewater generated during sulfide mineral processing. Microbial oxidation of the inorganic sulfur compounds can be exploited in a microbial fuel cell to generate an electrical current. Mixed cultures of acidophilic microorganisms from metal sulfide containing environments including sediment from an acid mine drainage stream and an acid sulfate soil were tested in a microbial fuel cell. The cultures were investigated for their ability to donate electrons from anaerobic tetrathionate oxidation to the anode, creating an electrical current that could be utilized for ferric iron reduction in the cathode. The oxidation of tetrathionate during current generation was coupled to an increase in sulfate in solution. An electrical current was also generated from the oxidation of thiosalts in a sulfide mineral processing wastewater. Microorganisms present in the mixed cultures were identified by high throughput next generation sequencing of the 16S rRNA gene and included autotrophic inorganic sulfur compound oxidizing Acidithiobacillus species as well as heterotrophic and ferrous iron oxidizing *Ferroplasma* spp. and *Sulfobacillus* spp. The coupling of inorganic sulfur compound oxidation to current generation in a microbial fuel cell technology may be suitable for bioremediation of sulfide mineral processing wastewaters.

Invited Lecture

L38

Sulfoquinovose degradation pathways in bacteria

David Schleheck

University of Konstanz, Germany

Sulfoquinovose (SQ, 6-deoxy-6-sulfoglucose) is the polar headgroup of the sulfolipid (SQDG) in all higher plants, mosses, ferns and algae, and in most photosynthetic bacteria. SQ represents a significant proportion of the organo-sulfur in nature and, thus, plays an important role in the biogeochemical sulfur cycle. There is evidence for at least two SQ-degradative pathways in bacteria, but no enzymic reaction or gene in any pathway has been defined, though a sulfoglycolytic pathway has been proposed. We found that *Escherichia coli* K-12, the most widely-studied prokaryotic model organism, is able to utilize SQ for growth. SQ is catabolized through "sulfoglycolysis", involving four newly discovered reactions that we established using purified, heterologously expressed enzymes: 6-deoxy-6-sulfoglucose (SQ) isomerase, 6-deoxy-6-sulfofructose (SF) kinase, 6-deoxy-6-sulfofructose-1-phosphate (SFP) aldolase, and 3-sulfolactaldehyde (SLA) reductase. The pathway yields dihydroxyacetone phosphate (DHAP), which powers energy conservation and growth of *E. coli*, and the sulfonate product 2,3-dihydroxypropane-1-sulfonate (DHPS), which is excreted. The corresponding SQ-gene cluster is found in >90% of the known *E. coli* genomes, and in a wide range of other Enterobacteriaceae, e.g., *Salmonella*, *Klebsiella* and *Pantoea* species. Hence, we presume that sulfoglycolysis plays a significant role in bacteria in the alimentary tract of all herbivores and omnivores, and in human and plant pathogens. We are currently revealing a second degradative pathway for SQ in a typical soil bacterium, *Pseudomonas putida* SQ1. This organism excretes 3-sulfolactate (SL) instead of DHPS during growth with SQ, and the present enzymic, proteomic and analytical-chemical data indicate that the pathway proceeds in analogy to the well-known Entner-Doudoroff pathway for glucose-6-phosphate in *Pseudomonas* species, through four novel enzymes: NAD-dependent SQ-dehydrogenase, 6-deoxy-6-sulfogluconolactone (SGL) lactonase, 6-deoxy-6-sulfogluconate (SG) dehydratase, 2-keto-3,6-dideoxy-6-sulfogluconate (KDSG) aldolase, and sulfolactaldehyde (SLA) dehydrogenase. The excreted SL and DHPS can be mineralized by other environmental bacteria through readily defined degradation pathways and desulfonative enzymes. Hence, a complete degradation of SQ can be accomplished by bacterial communities.

Invited Lecture

L39

The role of sulfur in marine methane oxidation

Jana Milucka

Department of Biogeochemistry, Max Planck Institute for Marine Microbiology, Bremen, Germany

Methane oxidation coupled to sulfate reduction is a major process responsible for reducing the emissions of methane, a potent greenhouse gas, from marine sediments. Sulfate-coupled AOM is believed to be performed by a microbial consortium of methanotrophic archaea and sulfate-reducing Deltaproteobacteria but the underlying mechanism remains a geomicrobiological puzzle. We proposed a new model for marine AOM, in which both methane oxidation and sulfate reduction to zero-valent sulfur is performed by the methanotrophic archaea. Furthermore, we could show that the associated Deltaproteobacteria are under AOM conditions capable of disproportionating the produced sulfur in a form of disulfide to sulfate and sulfide. These new observations expand the physiological diversity of known microbial sulfur metabolisms. Moreover, our results suggest that zero-valent sulfur plays a key role in AOM, which has important implications for biogeochemical carbon and sulfur cycling in marine sediments. In my talk, I will introduce our ongoing work on sulfur cycling associated with AOM in marine enrichment cultures, using a combination of molecular, microbiological and biogeochemical approaches. I will present our preliminary results from metagenomic and metatranscriptomic analyses of these enrichment cultures which provide first insights into the enzymatic mechanisms of sulfur-cycling associated with AOM.

L40

Sulfur disproportionation is achieved by co-metabolism with photosynthetic sulfide oxidation to sulfur

Naoki Kamiya, Katsumi Matsuura, Shin Haruta

Department of Biological Sciences, Tokyo Metropolitan University, Japan

We found a novel co-metabolism of sulfur disproportionation and photosynthetic sulfide oxidation to sulfur resulting in sulfate production from sulfide. Hot spring microbial mats we analyzed were dominated by green filamentous photosynthetic bacterium, *Chloroflexus aggregans* which anaerobically utilized sulfide as an electron donor and produced sulfur globule. However, the microbial mats anaerobically oxidized sulfide to sulfate. This study will present sulfur disproportionation in the mats has an important role of sulfur consumption and sulfide supply for photosynthetic bacteria as a primary producer. Elemental sulfur disproportionation is an anaerobic metabolism which utilizes elemental sulfur as both electron donor and acceptor, and produces sulfate and sulfide. Because produced sulfide decreases the energy efficiency, growth with this metabolism requires continuous sulfide removal such as abiotic sulfide precipitation with metal species. We are studying on sulfur metabolism of *C. aggregans*-dominating hot spring microbial mats. The mats developed at 65 degree Celsius were anaerobicaly incubated with sulfide as the sole sulfur and electron source. Sulfide concentration decreased with increase in sulfate concentration only in the light. This sulfate production was suppressed by the addition of molybdate, an inhibitor of ATP sulfurylase indicating sulfur disproportionation involved in sulfate production. We confirmed isolated *C. aggregans* did not produced sulfate as a result of sulfide consumption in the same condition, and previous genome research about *C. aggregans* indicates this bacterium oxidizes sulfide to most likely elemental sulfur. In order to confirm the existence of sulfur disproportionating bacteria, the mats were repeatedly cultivated in elemental sulfur disproportionating medium containing ferrihydrite as abiotic sulfide remover. Sulfide production was detected during the cultivation. Our results indicated *C. aggregans* acted as biotic sulfide scavenger in the microbial mats to help sulfur disproportionation. Growth of *C. aggregans* was also likely promoted through sulfur consumption and sulfide production by sulfur disproportionating bacteria.

Key Note Lecture

L41

Microbial sulfur metabolism and colorectal cancer risk

H. Rex Gaskins

Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign, USA

Despite the centrality of sulfur metabolism to most anaerobic microbial ecosystems, relatively little is known about the microbial assemblages and ecological constraints that contribute to microbial sulfur metabolism in the human colon. This gap in knowledge exists even with compelling evidence that bacterial-derived hydrogen sulfide is linked to prevalent human colonic disorders, namely inflammatory bowel disease (IBD) and colorectal cancer. Most of the attention given to the contribution of sulfidogenic microbes to colonic disorders has focused on sulfate-reducing bacteria (SRB), which are ubiquitously present in human colonic. Much less is known regarding the abundance of microbes capable of conserving energy through the utilization of organic sulfur sources in the human colon.

We are examining the extent to which bacterial-derived hydrogen sulfide may serve as a proinflammatory and genotoxic insult that modifies colon cancer risk. Data will be presented which demonstrate that the human colonic mucosa is persistently colonized by bacteria capable of generating sulfide from both inorganic and organic sulfur sources along with evidence that sulfide activates molecular pathways that underlie epithelial inflammation and hyperplasia, a phenotype common to both ulcerative colitis and colorectal cancer. Published studies will also be summarized, which demonstrate direct free radical based genotoxicity by exogenous sulfide that is independent of host cell metabolism. These observations highlight the possible role of bacterial-derived sulfide as an colonic insult that, given a predisposing genetic background, may lead to genomic instability or the cumulative mutations characteristic of colorectal cancer.

Abstracts of Posters

Abstracts are organized according to the session.

P1

Isotopic insights into the metabolism of sulfur disproportionation

Emma Bertran, David Johnston

Department of Earth and Planetary Sciences, Harvard University, USA

Microbial sulfur disproportionation imparts large, mass-dependent sulfur isotope effects that are preserved in modern marine and geological materials. For example, this metabolic pathway has been used to account for the discrepancy between the classic fractionation limit produced by pure cultures of sulfate reducers and the isotopic composition of sulfides in modern and paleoenvironments. Despite being attributed this crucial role, the biochemical underpinning and physiological controls on disproportionation that allow for such large fractionations are poorly constrained. In addition, recent work on the capacity of sulfate reduction to produce similarly large isotope effects has put into question the true role of disproportionation in the sulfur cycle, accentuating our general lack of understanding of this metabolic pathway. The very specific environmental niche occupied by disproportionation suggests even further that identifying a high fidelity isotopic biosignature would be of great utility. Here we present an early dataset with this longer-term goal. Starting with thiosulfate disproportionation, the multiple sulfur isotopic composition of substrate thiosulfate, and products sulfate and sulfide were tracked during the growth of *Desulfocapsa sulfexigens* in a series of batch experiments. The time series analysis and inclusion of minor isotopes allows added information about the isotopic effects associated with each specific step in the reaction network. These will be used to inform isotope models built to understand the broad role of disproportionation and oxidative pathways in the sulfur cycle.

P2

Motility of electric cable bacteria

Jesper Tataru Bjerg, Lars Riis Damgaard, Simon Agner Holm, Lars Peter Nielsen

Aarhus University, Denmark

Cable bacteria are filamentous sulfide oxidizers which can perform electrogenic sulfide oxidation using periplasmic structures capable of transmitting electrons over cm distances. All cable bacteria described so far couple sulfide oxidation with reduction of oxygen, nitrate, or nitrite and form a monophyletic cluster within Desulfobulbaceae. Growth studies and geochemical oscillations observed underneath photosynthetic mats have suggested that cable bacteria are motile and responsive to oxygen and/or sulfide. The aim of the present study was to characterize cable bacteria motility and to evaluate its role in establishing and optimize contact with oxic and sulfidic zones. Sediment enriched with cable bacteria was placed in microscope slide chambers that established a transparent transition zone from sulfidic sediment to an oxic-anoxic interface. Velocity, direction, and mode of cable bacterial motility were recorded by time-lapse microscopy. After analysis, the identity of a subset of cable bacteria was confirmed by fluorescence in situ hybridization. Cable bacteria exhibited gliding motility over surfaces and in sediment, with a maximum velocity of 2.2 μm s^{-1}, and a mean of 0.48 μm s^{-1}. Sections of the cable bacteria frequently formed loops and moved for extended periods with loop sections first rather than tip first. The filaments apparently rotated around their longitudinal axis, evidenced by loops tending to twist when parts of a filament were not attached to a surface. Cable bacteria moved partially into oxic water and curled up near the oxic-anoxic interface with part of the filament trailing back to the sediment. We propose that motility serves to position the cables with an optimal balance between numbers of cells in cathodic and anodic zones and the minimum number of current bearing cells in between. The mechanism and cell-cell communication underlying the observed motility are still to be resolved.

P3

Cellular and molecular mechanisms underpinning sulfur isotope fractionation in sulfate reducing bacteria

Alexander Bradley[1], William Leavitt[1], David Johnston[2], et al.

[1] *Washington University in St. Louis, Saint Louis, MO, USA*
[2] *Harvard University, Cambridge, MA, USA*

In this study we focus on the cellular and molecular level mechanisms of isotope fractionation during dissimilatory sulfate reduction. At the cellular level, we have examined the concentration dependence of sulfur isotope fractionation in two model sulfate reducing bacteria: the freshwater strain *Desulfovibrio vulgaris* str. Hildenborough and the brine strain *Desulfovibrio alaskensis* G20. Using continuous culture devices we have grown each strain under constant growth rates, varying sulfate concentration from 0.1 to 10 mM. Each relationship can be fit with a Monod curve, but the fitted constants differ markedly between strains. In previous work we held sulfate concentrations constant and examined the relationship between sulfur isotope fractionation, and sulfate reduction rates over a wide range of rates – the resulting relationship is a hyperbolic fit between rate and fractionation.

We have combined these results in a model framework in which we represent the magnitude of sulfur isotope fractionation as a function incorporating rates of electron donor supply and sulfate supply to the cellular machinery responsible for transformation of sulfate to sulfide, and account for strain specific factors, such as sulfate and electron donor affinity constants.

These results are best understood by casting them into an enzymatic reaction network in which fluxes and fractionations may be imposed at any given step. In combination with recent work aimed at uncovering enzyme-specific sulfur isotope fractionations, this moves us towards an understanding of the biological underpinnings of sulfur isotope fractionation, and the genetic variations that may impose phenotypic differences between strains. We consider the effects of selective pressure on the evolution of sulfate and electron acquisition machinery over the course of evolutionary and Earth history. These will aid efforts to reconstruct ambient sulfate concentrations from sedimentary sulfur isotopic compositions.

P4

The impact of environmental conditions on sedimentary $\delta^{34}S$ records: rethinking the evolution of the microbial sulfur cycle

David Fike[1], Catherine Rose[1, 2], Robert Aller[3]

[1] Department of Earth & Planetary Sciences, Washington University, St. Louis, MO 63130, USA
[2] Department of Geology, Trinity College Dublin, Dublin 2, Ireland
[3] School of Marine & Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794 USA

Sulfur isotope ratio data ($\delta^{34}S$) have been used to provide significant insights into global biogeochemical cycling over Earth history, providing a framework for reconstructing both global redox budgets and microbial metabolic activity. However, as the record of ancient oceanic conditions becomes better resolved, reports of coeval but divergent isotopic proxies are becoming increasingly common. These sulfur isotope records are characterized not just by divergent $\delta^{34}S$ values, but also by differences in the spatial signature and magnitude of isotopic variability. Such discordant data suggest that we do not fully understand how isotopic signatures are incorporated and eventually preserved in the rock record. Here we examine the spatial signature and magnitude of isotopic variability in modern marine systems as a function of depositional environment and differential microbial metabolic activity. Varying depositional conditions, particularly sedimentary reworking, are seen to play a major role in generating and modifying the isotopic signatures of sulfur phases in modern environments. These observations can be extrapolated to investigate records of sulfur cycling in ancient strata. The results suggest that many apparent secular $\delta^{34}S$ trends may be related to changing depositional environment rather than changes in the global sulfur cycle – with implications for how we infer microbial metabolic activity from sulfur isotopic records. Together, these observations provide new insights that enable us to reflect on and refine our interpretations of chemostratigraphic $\delta^{34}S$ data that have the potential to constrain the behavior of the microbial sulfur cycle over geological timescales.

P5

The kinetics and environmental significance of sulfide oxidation by a novel green sulfur bacterium isolated from a stratified estuary

Alyssa J. Findlay, Thomas E. Hanson, George W. Luther III

University of Delaware, College of Earth, Ocean, and Environment, USA

The oxidation of hydrogen sulfide is an important part of the sulfur cycle that is microbially mediated in many environments. Field studies were conducted during the summers of 2011- 2014 in the Chesapeake Bay, USA during which the redox chemistry and microbiology of the stratified water column were characterized. The redox conditions, bottom water sulfide concentrations, and location of the pycnocline varied between years. In 2011, 2013, and 2014, phototrophic sulfide oxidizing bacteria (PSOB) were enriched from waters sampled at and below the oxic/anoxic interface, and in 2012, 2013, and 2014, light dependent sulfide loss was observed in freshly collected anoxic water column samples. Extremely low levels of light (0.01 – 5 μEi) were found to cause 2-10 fold increases in sulfide loss over that observed in dark incubations. Laboratory experiments conducted with enrichment cultures of PSOB from the Chesapeake Bay constrained their sulfide oxidation kinetics under varying light and sulfide conditions. These experiments indicate a value for Ks of 10 μM and a Vmax of 50 μM/min/mg protein for the oxidation of sulfide. Phototrophic activity becomes light saturated over 5 μEi, and a significant uptake rate is observed under dark conditions. Oxidation products include nanoparticulate elemental sulfur and polysulfides. These results are compared with similar experimental determination of sulfide oxidation kinetics in Chlorobaculum tepidum and RSC1 (a green sulfur bacterium isolated from a Bahamian sink hole). Finally, using these results from the field and lab, a productivity model for PSOB in the Chesapeake Bay was developed, which indicates that PSOB play an important, yet variable, role in regulating sulfide oxidation at the pycnocline in the Bay.

P6

Distribution of electric fields generated by electrogenic sulfide oxidation in Aarhus Bay sediment

Diego Giao[1, 2], Nils Risgaard Petersen[1, 2], Lars Peter Nielsen[1, 2]

[1] Section for Microbiology, Department of Bioscience, Aarhus University, Ny Munkegade 114-116, 8000 Aarhus C, Denmark
[2] Center for Geomicrobiology, Department of Bioscience, Aarhus University, Ny Munkegade 114-116, 8000 Aarhus C, Denmark

Cable bacteria are long filamentous bacteria of the Desulfobulbaceae family, which are able to create centimeter-deep suboxic zone in marine sediment by coupling distant H_2S oxidation to O_2 reduction (1, 2) The long distance electron transfer between these redox reactions creates measureable electric fields, which reflect the rates and locations of cable bacteria activity in the sediment. We aim to describe the in situ centimeter-scale heterogeneity of the biogenic electric fields in Aarhus Bay, and the correlations with the distribution of cable bacteria and sulfide sources.

We employed the new electric potential microsensor in box cores of intact sediment to calculate the current densities associated to the biogenic electric fields and their spatial distribution. We also measured the depth distribution of cable bacteria and FeS, as they are potentially the best proxies to understand the in situ distribution of biogenic electric fields.

The activity and cable bacteria were very heterogeneously distributed with current densities varying from 6 to 398 mA m^{-2} among spots only few centimeters apart. These range of current densities are comparable to the ones we measured in Tokyo Bay (Japan), and to rates calculated from geochemical data in The Netherlands and the North Belgium coast (3).

This is the first report of the in situ presence of cable bacteria in Aarhus Bay and their electric fields associated to electrogenic sulfide oxidation. Our results show a high spatial heterogeneity that we do not observe in experiments with homogenized sediment. This suggests that in situ processes like bioturbation, could be key players in the distribution of electrogenic sulfur oxidation by cable bacteria.

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[2] Pfeffer, C., et al (2012). Nature 491, 218–221.

[3] Malkin, S.Y., et al (2014). ISME J 1–12.

P7

Geochemical gradients in marine sediment are reflected in the community composition of sulfate-reducing microorganisms

Lara M. Jochum[1], Lars Schreiber[1], Alexander Loy[2, 3], Claus Pelikan[2], Bo Barker Jørgensen[1], Andreas Schramm[1, 4], Kasper U. Kjeldsen[1]

[1] *Center for Geomicrobiology, Department of Bioscience, Aarhus University, Aarhus, Denmark*
[2] *Department of Microbiology and Ecosystem Science, Division of Microbial Ecology, Faculty of Life Sciences, University of Vienna, Vienna, Austria*
[3] *Austrian Polar Research Institute, Vienna, Austria*
[4] *Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus, Denmark*

Sulfate reducing microorganisms (SRM) are key drivers of anaerobic organic matter mineralization in marine coastal sediments and thereby represent an important link between the marine sulfur and carbon cycles. Marine sediments are populated by an uncultured diversity of SRM and the factors controlling their vertical distribution remain largely unknown. Using qPCR and high throughput sequencing of dsrB, a functional marker gene for SRM, we aimed to relate SRM community structures to distinct geochemical zones of coastal sediment.

We analyzed samples from the sediment surface, the underlying sulfate-rich zone, the Sulfate-Methane-Transition-Zone (SMTZ: the depth at which sulfate becomes depleted and methanogenesis takes over) and the methanogenesis zone from four stations in Aarhus Bay at which the SMTZ occurred at different depths.

According to qPCR the relative abundance of SRM compared to the total microbial population decreased from 5-17% in surface sediments to 1-2% in deep methanogenic sediments depending on the station. The most striking difference between any two geochemical zones was a 50% or higher decrease in abundance from surface sediments to the sulfate-rich zone. Comparative qPCR assays of *dsrB* and *aprA* (another functional marker gene for SRM) showed similar results for surface sediments, while *dsrB* was more abundant than *aprA* in the zones below. This suggests that a proportion of *dsrB*-carrying microorganisms do not carry *aprA* genes and thus cannot conserve energy by sulfate reduction. To describe community structures we analyzed on average 40,000 *dsrB* reads per sample.

The *dsrB* diversity dropped with sediment depth. Distinct *dsrB*-gene variants were associated with the surface and sulfate-rich sediments, while deeper zones accommodated more similar communities. However, those were composed of members that are only distantly related to known SRM. In conclusion, abrupt SRM community shifts occur at transitions between the sediment surface, the sulfate-rich zone and the SMTZ where sulfate is depleted.

P8

Seasonal variations in concentrations and isotopic composition of sulfur species in a low-sulfate, warm, monomictic lake

Alexey Kamyshny[1], Nadav Knossow[1], Werner Eckert[2], Alexandra Turchyn[3], Gilad Antler[3]

[1] *Ben-Gurion University of the Negev, Beer Sheva, Israel*
[2] *Israel Oceanographic & Limnological Research Ltd., Migdal, Israel*
[3] *Department of Earth Sciences, University of Cambridge, United Kingdom*

The main goal of this research was to study the annual variability of the concentrations and the isotopic composition of main sulfur species and sulfide oxidation intermediates in the water column of Lake Kinneret, monomictic fresh-water lake, in which sulfate concentrations are below 1 mM that is similar to concentrations proposed to have existed in the Paleoproterozoic ocean. At the deepest point of the lake, the sulfate inventory decreases by more than 20% between March and December due to microbial sulfate reduction leading to the buildup of hydrogen sulfide. Hydrogen sulfide inventory in the water column increases from May to December, and sharply decreases during the lake mixis in January. At the initial stages of stratification, sulfur isotope fractionation between sulfate and hydrogen sulfide is low (11.6‰) and sulfur oxyanions (e.g. thiosulfate and sulfite) are the main products of the incomplete oxidation of hydrogen sulfide. During the stratification and at the beginning of the lake mixing (July – December), sulfur isotope fractionation increases to 30±4‰ in October. During the erosion of the chemocline, zero-valent sulfur prevails over sulfur oxyanions. In the terminal period of the water column mixing (January), the inventory of sulfide oxidation intermediates increases, and sulfur isotope fractionation decreases to 20±2‰. Sulfur isotope fractionation between sulfate and hydrogen sulfide as well as concentrations of sulfide oxidation intermediates can be explained either by microbial sulfate reduction alone or by microbial sulfate reduction combined with microbial disproportionation of sulfide oxidation intermediates. No clear positive correlation between concentrations of sulfide oxidation intermediates in the water column and sulfur isotope fractionation between sulfate and hydrogen sulfide was observed.

P9

Degradation of carbonyl sulfide, an atmospheric sulfur compound, by Actinobacteria and fungi

Yoko Katayama[1], Takahiro Ogawa[2], Hiromi Kato[3]

[1] *Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Japan*
[2] *Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Japan*
[3] *Graduate School of Life Science, Tohoku University, Japan*

Soil environment has been considered as a major sink of carbonyl sulfide (COS). COS is the most abundant sulfur compound in the troposphere and is an important source of the stratospheric sulfate aerosol. Therefore, COS influences the Earth's radiation balance and ozone chemistry. COS is also a greenhouse gas because of its high global-warming potential. Soil microorganisms has been considered to play the important roles in the process of sink of atmospheric COS and the enzymes such as COS hydrolase has been isolated from chemolithoautotrophic *Thiobacillus thioparus*. However, distribution of microorganisms contributing to the degradation of COS is still unknown. In this study, we report the COS degradation by chemoorganotrophic soil microorganisms such as Actinobacteria and fungi for better understanding the major soil microorganisms in degradation of the atmospheric COS.

Total 43 strains of Actinobacteria that is covering 8 major taxonomic groups in this phylum were obtained from the culture collection or isolated from soil, and the COS degrading ability were examined. Within these, 36 strains harbored the ability, in which *Dietzia maris* showed the highest activity. Fungal strains isolated from the forest soils were phylogenetically identified based on the gene sequence analysis of the ITS region. Total 24 fungal strains out of 42 isolates showed degradation of COS, in which 11 isolates can degrade 30 ppmv COS to less than the detection limit within 1.5 h's incubation. *Trichoderma* spp. showed the highest COS degrading activity in the fungal isolates examined. These results indicate that COS degrading ability can be find out with high frequencies in Actinobacteria and fungi and these microorganisms share the important roles in degradation of atmospheric COS in soil environment.

P10

The role of electron-bifurcating transhydrogenase in setting lipid H-isotope ratios in bacterial sulphate reducers

William Leavitt[1], Melanie Seuss[1], Magdalena Osburn[2], Alexander Bradley[1]

[1] Washington University in St. Louis, Department of Earth & Planetary Sciences, St. Louis MO, USA
[2] Northwestern University, Department of Earth & Planetary Sciences, Evanston, IL, USA

Recent studies have shown that lipids of bacterial sulphate reducers (BSRs) are strongly depleted in deuterium relative to growth water (-250‰) (1). In aerobic microorganisms, the deuterium to hydrogen (D/H) ratio relative to growth water varies systematically with central C metabolism (2). However, this pattern does not hold in sulphate reducers (1). Moreover, we have a poor understanding for the mechanism(s) by which these isotopic signatures are imparted during lipid biosynthesis. Recent work in aerobic methylotrophs (3) implicates transhydrogenase activity as a critical control on lipid D/H. Transhydrogenases are a class of oxidoreductase enzymes that are responsible for transferring reducing power between intracellular pools of pyrimidine nucleotides. At least three classes of transhydrogenases have been described: i) proton translocating transhydrogenase PntAB, which is known to carry an extremely large hydrogen isotope fractionation (4); ii) soluble energy-independent transhydrogenase UdhA, present in many proteobacteria; iii) electron-bifurcating transhydrogenase NfnAB, present in many anaerobic bacteria and archaea. Here we focus on determining the role of NfnAB-2 in controlling the D/H lipids in *Desulfovibrio alaskensis* strain G20. Utilizing mutant strains from a transposon library (5), we have shown NfnAB-2 plays a large role in inducing fractionation between lipid and water under some growth conditions. We discuss the implications for understanding H-isotope fractionation during microbial fatty acid biosynthesis in BSRs, anaerobes in general, and as a sedimentary microbial metabolic tracer.

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P11

Multiple S isotope biosignatures in Aarhus Bay sediments

Andrew Masterson[1], Marc Alperin[2], Gail Arnold[3, 4], Will Berelson[5], David Johnston[1]

[1] *Harvard University, USA*
[2] *Univ. of North Carolina, Chapel Hill, USA*
[3] *Dept. of Geological Sciences, Univ. of Texas, El Paso, USA*
[4] *Center for Geomicrobiology, Aarhus University, DK*
[5] *Dept. of Earth Sciences, Univ. of Southern California, USA*

The connection between rates of microbial sulfate reduction (MSR) and the magnitude of an expressed fractionation $^{34}\varepsilon = \delta^{34}SSO_4 - \delta^{34}SH_2S$ in continuous cultures of sulfate reducers has recently been extended to include ^{33}S (Leavitt et al. 2013). The implications of that finding for S isotope signatures in marine sediments, however, remains to be explored. Theoretically, dramatic changes in organic matter reactivity towards sulfate reduction would be reflected in the pore water sulfate isotope profile, caused by changes in the rates of MSR at the metabolic scale. The purpose of this study is two-fold (1) to ascertain whether intrinsic changes in cellular-level rates of sulfate reduction are required to reproduce the minor isotope signatures in pore water sulfate from a shallow water environment, and (2) to determine how solid phase sulfides reflect the mass balance of pore water sulfide. We have explored both by carrying out the full S isotope and geochemical characterization of a gravity core from Aarhus Bay, Denmark.

Aarhus Bay (Site M1) displays an approximately linear sulfate concentration profile, and there is a consistent ~66‰ offset between coeval pore water SO_4^{2-} and H_2S, and clear closed system behavior observed in the minor isotope ($\Delta^{33}S$) signatures, however, sedimentary pyrite ($\delta^{34}SFeS_2$, $\Delta^{33}SFeS_2$) exhibits little downcore variability. Diagenetic modeling of the pore water sulfate profile demonstrates that little change in intrinsic fractionation characteristics of MSR are required to reproduce the profile. Furthermore, the lack of strong downcore variation in pyrite isotope signatures implies that, while the pore water sulfur species display strong closed system behavior, solid phase sulfides do not strongly inherit those characteristics.

Leavitt W. D., Halevy I., Bradley A. S. and Johnston D. T. (2013) Influence of sulfate reduction rates on the Phanerozoic sulfur isotope record. Proc. Natl. Acad. Sci. U. S. A. 110, 11244–9.

P12

The sulphur cycling and sulphide formation in deep groundwater bedrock

Hanna Miettinen, Minna Vikman, Kaisa Marjamaa, Heikki Salavirta, Merja Itävaara

VTT Technical Research Centre of Finland Ltd, Finland

Sulphur is among the most abundant elements on Earth. It is mainly present as pyrite or gypsum in rocks and sediments and as sulphate in seawater. In Finland there are areas where the deep subsurface ground water has a sulphate rich layers originating from the old Littorina Sea, the predecessor of the Baltic Sea. In some places sulphide formation in sulphate rich water is underway whereas in other similar circumstances sulphide formation is not measurable. This study focuses on sampling three different deep subsurface (100 to 400 m bsl) ground water types situated near each other: Sulphate and sulphide rich groundwater, sulphate rich groundwater and salty groundwater. The aim is to sequence the different type of water samples for metapathways related to sulphur cycle in DNA and mRNA fractions to compare the differences between the samples and to discover pathways to sulphide formation. Preliminary results show that the amount of total number of cells in different ground waters vary between $3.5 \cdot 10^4$ to $4.7 \cdot 10^5$ mL^{-1}, the highest cell counts found in the ground water with high sulphide and sulphate concentration and the lowest count found in the salty groundwater.

P13

Sulfate-reducing bacteria in Mediterranean lagoons: similarities and disparities between the different biogeographic areas

Christina Pavloudi[1, 2, 3], Anastasis Oulas[1], Katerina Vasileiadou[1, 4], Christos Arvanitidis[1]

[1] *Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, 71500 Heraklion, Crete, Greece*
[2] *Department of Biology, Faculty of Sciences, University of Ghent, 9000 Ghent, Belgium*
[3] *Department of Microbial Ecophysiology, Faculty of Biology, University of Bremen, 28359, Bremen, Germany*
[4] *Department of Biology, University of Patras, 26504 Rio, Patras, Greece*

Lagoons are naturally enriched habitats, with unstable environmental conditions caused by their confinement from the sea and their shallow depth. Such ecosystems are characterized by increased hypoxia and high concentrations of hydrogen sulfide. The aim of the present study was to examine the sulfate reducing bacterial community in the lagoonal sediments of the Amvrakikos Gulf (Ionian Sea, Western Greece) and to compare it with the communities reported from other Mediterranean lagoons.

For this purpose, sediment samples were collected from five lagoons, located in Amvrakikos Gulf (Ionian Sea, Western Greece). In each lagoon, two sampling stations were chosen, with different connectivity to the sea. DNA was extracted from the sediment upper layer (0-2cm) and was further processed through next generation sequencing (454 GS FLX Titanium Series, Roche) of the V5-V6 region of the 16S rRNA gene and of a region of the dissimilatory sulfite reductase (dsr) gene. Moreover, the dsr sequences were processed with the SEQenv pipeline and were thus annotated with environment descriptive terms occurring in the relevant literature. Preliminary results indicate that sequencing of the dsr gene, which is found in sulfate reducing bacteria, provides in depth information regarding this particular phylogenetic group which is not provided by the commonly used 16S rRNA. Furthermore, our results indicate that ecosystem function changes in response to the geochemical variables fluctuation as microbial diversity in the stations closer to the sea varies from the one in the stations located inside the lagoons. In addition, each lagoonal community is annotated with different environmental descriptive terms, at least as their abundance is concerned, which may indicate that the communities have, to some extent, different sources of origin.

P14

Sulfur isotope fractionation during evolution experiments with sulfate reducing microbes: physiological controls and genetic associations

Andre Pellerin[1], Jyotsana Singh[1], Eric Collins[2], Luke Anderson-Trocme[1], Boswell Wing[1]

[1] *Dept. of Earth and Planetary Sciences, McGill University, Canada*
[2] *School of Fisheries and Ocean Sciences, University of Alaska Fairbanks, USA*

Sulfur isotope fractionation during dissimilatory sulfate reduction is controlled by the energy metabolism of sulfate reducing microorganisms. While this metabolism responds to variability in the local environment, it is ultimately dependent on the underlying genotype. However, the basic interplay between microbial evolution, which determines genotype, and S isotope fractionation has not been examined. We investigated the evolutionary response of S isotope fractionation in *Desulfovibrio vulgaris* Hildenborough (DvH) and *Desulfomicrobium baculatum* (Dbac) through experimental evolution. Replicate lines of DvH and Dbac were serially transferred in batch cultures for up to 1000 generations. Both the descendant DvH and Dbac strains were more fit than their ancestors with 20% enhancement in realized growth rates for DvH and 300% enhancements for DBac. In-situ monitoring of population size showed that these fitness changes largely reflected improvements in maximum growth rates. Ancestral cultures of DvH respired more rapidly than ancestral cultures of DBac, and produced sulfide that was slightly less depleted in $^{34}S/^{32}S$ relative to sulfate (-7.0±1.3‰ for DvH; -15.4±0.7‰ for Dbac). When the isotope assay was repeated on the evolved lines, Dbac populations reproducibly showed a lower $^{34}S/^{32}S$ fractionation than their ancestors (-12.3±0.4‰). On the other hand, evolved DvH populations displayed very similar fractionations to their ancestors. As illustrated here changes in S isotope fractionation during evolutionary adaptation of growth rate mimic, in a broad sense, known physiological responses of S isotope fractionation to growth within, and between strains of dissimilatory sulfate reducers. As a result, it may be possible to disentangle metabolic and environmental effects imprinted by the sulfate reducing metabolism in natural environments. Community-level metagenome sequencing of Dbac ancestor and evolved populations is underway to explore the expression changes that are associated with the evolutionary shifts described here, potentially pointing to a mechanistic bridge between genotype and phenotype.

P15

Benthic sulfur cycling in the oligotrophic and oligohaline Bothnian Bay

Bo Thamdrup, Marianela Fallas Dotti, Emma Hammarlund, Amelia-Elena Rotaru, Hannah Sophia Weber

Nordic Centre for Earth Evolution, Department of Biology, University of Southern Denmark

The northernmost basin of the Baltic is characterized by salinities below 5, low productivity, high riverine inputs of particulate Mn and Fe, and sparse infauna. These conditions make the sediments relevant for studying microbial sulfur transformations and their interactions with C, Mn and Fe cycling at low sulfate concentrations. The biogeochemistry of Bothnian Bay sediments is poorly explored but important for understanding the basin's role in the Baltic system, e.g., as a phosphorus sink. At the same time, its investigation may provide insights to marine biogeochemistry in early, sulfate-poor oceans.

With this dual aim, we determined the depth distribution of sulfur species, their isotopic composition, sulfate reduction rates, and pathways of anaerobic carbon oxidation at several stations in the Bothnian Bay. Sulfate reduction accounted for 35–60 % of carbon oxidation, being suppressed by Mn and Fe reduction near the surface. Sulfate penetration varied from 10 to 40 cm between sites, and secondary peaks of sulfate reduction were located near the depth of depletion, indicating anaerobic methane oxidation as a major sulfate sink. This link was further supported by the distribution of methane. The isotopic composition, $\delta^{34}S$, of reduced iron sulfides accumulating in the sediment was ~60 ‰ lighter than porewater sulfate, indicating a strong fractionation despite relatively low sulfate concentrations. Such high fractionation may result from the combined effects of sulfate reduction and sulfur disproportionation in the Mn- and Fe-rich sediment, in which H_2S was generally not detectable. By contrast, fractionation factors derived from inverse modelling of profiles of concentrations and $\delta^{34}S$ of sulfate indicate fractionation of only ~15‰. Direct determinations of fractionation during sulfate consumption are underway to resolve the discrepancy between fractionation factors. Our results demonstrate that the Bothnian Bay provides an excellent setting for exploring sulfur dynamics and sulfate dependent methane oxidation under low sulfate conditions.

P16

Expression analysis of sulfide oxidizing enzymes and characterization of flavocytochrome-*c* sulfide dehydrogenase in a purple sulfur photosynthetic bacterium

Tímea Balogh[1], Ágnes Duzs[1], Enikő Kiss[1], András Tóth[1, 2], Gábor Rákhely[1, 2]

[1] Department of Biotechnology, University of Szeged, Szeged, Hungary
[2] Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Anoxygenic photosynthesis of purple sulfur bacteria requires reduced sulfur compounds as electron donors. Oxidation of sulfide is mediated by flavocytochrome-*c* sulfide dehydrogenase (Fcc) or, just like in colonic mucosa of human gut, by sulfide:quinone oxidoreductase (Sqr). Resultant electrons are passed into the photosynthetic electron transport chain at various points. Fcc is a periplasmic enzyme, donates electrons to small periplasmic *c*-type cytochromes, while Sqr is membrane associated and transfers electrons from sulfide oxidation directly to the quinone pool. There are relatively few experimental data on transcriptional characteristic of enzymes, participating in sulfur oxidation. Moreover, individual functions of Fcc and Sqr proteins are still to be understood. *Thiocapsa roseopersicina* is a phototrophic purple sulfur bacterium. There are three genes in the genome of *T. roseopersicina* BBS encoding enzymes potentially involved in the oxidation of sulfide: a flavocytochrome-*c* sulfide dehydrogenase (FccAB), and two different kinds of sulfide:quinone oxidoreductases (SqrD and SqrF). In order to investigate the function of above mentioned sulfide oxidases, effect of sulfide on the expression of *fcc*, *sqrD* and *sqrF* genes was characterized in *T. roseopersicina* BBS. Furthermore, for biochemical analysis of flavocytochrome-*c*, affinity tagged recombinant enzyme was expressed in *T. roseopersicina*. Subunits of the enzyme were purified by affinity chromatography and its kinetic features were determined. The observed transcriptomic responses indicate that Fcc catalyzes sulfide oxidation predominantly in the presence of low sulfide concentration, while sulfide:quinone oxidoreductases play role during growth in a media containing sulfide in higher amount. UV-visible absorption spectroscopy – used for further investigation of Fcc – resulted peaks, that are characteristic for redox active flavin prosthetic groups and, by measuring in vitro activity of purified recombinant Fcc, we were proved it catalyzes sulfide dependent cytochrome c reduction. Biochemical and kinetic properties of the enzyme were determined and these findings absolutely correlate with experimental data of transcriptional analysis.

P17

Searching for structures supporting electrogenic sulfur oxidation

Andreas Bøggild[1], Poul Nissen[1], Lars Peter Nielsen[2], Thomas Boesen[1]

[1] *Department of Molecular Biology and Genetics, Aarhus University, Denmark*
[2] *Department of Bioscience, Aarhus University, Denmark*

Cable bacteria, a filamentous member of the Desulfobulbaceae, were first described in 2012 in marine sediment from Aarhus Bay (Pfeffer et al.), but have since been found at other sites around the world (Malkin et al.). Their presence in sediments causes a distinct set of depth profiles for pH, oxygen and hydrogen sulfide, which can be explained by long distance electron transport along the multicellular filaments, enabling electrogenic sulfur oxidation in the anoxic environment centimeters below the sediment surface.

Though the electron transport can easily be observed indirectly in sediment cores, we still lack an explanation of how they are transported along the bacterial filament. Electron transport has been observed over micrometer distances in other organisms utilizing "nanowires", pili or pili-like appendages over, where electron transport has been suggested to be facilitated either directly by pilin subunits or by multiheme cytochromes organized along the nanowire (Gorby et al.). Cable bacteria could utilize similar structural arrangements or perhaps even a completely novel way of biological electron transport.

We are working on elucidating the subcellular structures responsible for the electron transport in the cable bacteria using electron microscopy of the multicellular filaments, while mass spectrometry, bioinformatics and other molecular biology and biophysical methods are employed to identify components at the molecular level.

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Pfeffer, Christian et al. "Filamentous Bacteria Transport Electrons over Centimetre Distances." Nature 491.7423 (2012): 218–221. Web. 2 Nov. 2012.

P18

Targeting sulfur assimilation in the development of new antibiotics: towards the identification and validation of dual CysK/CysM inhibitors

Barbara Campanini[1], Roberto Benoni[2], Thelma Pertinhez[3], Stefano Bettati[2], Marco Pieroni[1], Giannamaria Annunziato[1], Claudia Beato[1], Gabriele Costantino[1], Andrea Mozzarelli[1]

[1] *Dipartimento di Farmacia, Università di Parma, Italy*
[2] *Dipartimento di Neuroscienze, Università di Parma, Italy*
[3] *Centro Interdipartimentale Misure, Università di Parma, Italy*

Recent evidence indicates that blocking cysteine biosynthesis represents a valuable strategy to diminish bacterial resistance to oxidative stress and hence reduce persistence inside the host and increase antibiotic susceptibility (1-3). O-acetylserine sulfhydrylase catalyzes the last step of cysteine biosynthesis in bacteria and, being absent in mammals, represents a potential target for specific inhibitors. However, pathogens like *Salmonella typhimurium* possess two isoforms of this enzyme, CysK and CysM, whose respective roles during infection and persistency are not fully understood. So far, CysM has been an elusive target for inhibitors development and most of the molecules shown to be effective on CysK are much less effective on CysM (4,5). Fifteen cyclopropanecarboxylic acid derivatives were synthesized and tested on CysK and CysM. The activity of the compounds was measured by a fluorimetric binding assay, to estimate Kds for the enzyme, and by a 96-well plate activity assay in the presence of O-acetylserine and bisulfide, to calculate IC50s and identify non-competitive inhibitors. The structural determinants of binding have been evaluated by STD-NMR. With one exception, all the molecules bind to both CysK and CysM. Differently from previous observations (5), substituents that increase affinity to CysK also increase affinity to CysM. The best hit inhibits both isozymes and has a Kd of 60 ± 7 nM for CysK and 1.65 ± 0.06 microM for CysM. The biological activity of the best inhibitor on E. coli grown in minimal medium is being assessed both in liquid culture and on agar plates.

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P19

Metabolic and physiologic investigations of phototrophic purple sulfur bacteria in vitro and in situ through flow cytometry

Francesco Danza[1, 2], Mauro Tonolla[1, 2]

[1] *Microbial Ecology Group, Microbiology Unit, Plant Biology Department, University of Geneva, CH-1211 Geneva 4, Switzerland*
[2] *Laboratorio Microbiologia Applicata, Dipartimento Ambiente, Costruzione e Design, Scuola Universitaria Professionale della Svizzera Italiana (SUPSI), 6500 Bellinzona, Switzerland*

Phototrophic purple sulfur bacteria (PSB) are described as anaerobic anoxygenic photo-organisms that utilize reduced sulfur compounds like sulfide (H_2S) as electron donor and light as energy source. In batch cultures the predominant photolithoautotrophic metabolism is stimulated through light irradiation and H_2S supply. However the PSB natural environment, like the chemocline of Lake Cadagno (Switzerland), is subjected to continuous fluctuations. Consequently PSB need alternative metabolic strategies. In this sense, inclusion bodies of elemental sulfur and reduced carbon polymer like polyhydroxybutyrate (PHB) are essential. Aim of this study is to investigate the dynamic and ecological significance of inclusion bodies for PSB *Thiodictyon syntrophicum* Cad16 and *Chromatium okenii*. The application of flow cytometry (FC) for the characterization of physiological behavior of natural pigmented cells is a powerful technique allowing a rapid evaluation of PSB cell activity and dynamic description of intracellular inclusion bodies formation and depletion. Sideward and forward light scatter (SSC, FSC) values are linked with sulfide-oxidation and PHB biosynthesis reactions. Intracellular sulfur globules S^0 during light- oxidation of H_2S increase the internal cell complexity with a consequent rise of the SSC value. Decrease of SSC during dark period corresponds to a reduction of intracellular sulfur inclusions. A similar light/dark dependence is observed with FC for PHB. These observations suggest the relevance of inclusions for PSB metabolism during dark period. Moreover a rapid kinetic reactions evaluation is also possible. Under laboratory conditions *C. okenii* showed a faster H_2S oxidative activity compared to other tested PSB strains. Similarly *C. okenii* rapidly reacted to sulfide addition during in situ experiment. Through FC characterization of PSB culture we have a tool for rapid description of PSB population activity in situ. The idea is to describe in situ the fate of a single PSB population over long time, considering the influence of external biotic and abiotic factors.

P20

Catalytic properties of a type VI sulfide quinone oxidoreductase

Ágnes Duzs[1], András Tóth[1, 2], Brigitta Németh[2], Viven Tejsi[2], Tímea Balogh[1], Gábor Rákhely[1, 2]

[1] Department of Biotechnology, University of Szeged, H-6726 Szeged, Hungary
[2] Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, H-6726 Szeged, Hungary

Despite its toxicity sulfide involves in a variety of important physiological processes for instance functional in human digestive system, neurotransmitter in mammals or electron donor for photosynthetic sulfur bacteria. Flavocytochrome *c* (Fcc) and sulfide quinone oxidoreductase (Sqr) are ancient flavoproteins, members of the disulfide oxidoreductase enzyme family, which catalyse the oxidation of sulfide due to regulation of its concentration or electron release from this reduced sulfur compound. The photosynthetic purple sulfur bacterium *Thiocapsa roseopersicina* can utilize various reduced inorganic sulfur compounds (eg. sulfide) donating electrons for photolithoautotrophic growth. Three genes encoding sulfide oxidizing disulfide oxidoreductases were identified in the genome sequence: *fcc*, *sqrD* and *sqrF*. SqrD and SqrF belong to group IV and VI of the sulfide quinone oxidoreductase type enzymes, respectively, which proteins have not been characterized in detail yet. For biochemical analysis the SqrF protein fused to Strep II affinity tag was expressed in *T. roseopersicina*. The recombinant membrane-bound SqrF was successfully purified from membrane fraction to homogeneity by affinity chromatography. UV-vis absorption spectra of oxidized and reduced forms of purified SqrF protein was recorded. Based on the changes of characteristic peaks the covalently bound FAD cofactor is redox active in the pure protein. The recombinant SqrF enzyme catalyzes sulfur-dependent quinone reduction and prefers ubiquinone-type quinone compounds as electron acceptor. Effect of pH and temperature on the SqrF activity were examined. Furthermore, kinetic parameters of the enzyme for sulfide and quinone substrates were determined. The biochemical and kinetic analysis of the studied type VI sulfide quinone oxidoreductase highlighted that the affinity of this enzyme for sulfide is low, which propose that SqrF could play role in the oxidative sulfide metabolism of purple sulfur bacteria at high sulfide concentration conditions.

P21

Genome sequence of an uncultivated population of the purple sulfur bacterium *Chromatium okenii*

Niels-Ulrik Frigaard[1], Nicole R. Posth[2], Raymond P. Cox[3]

[1] *Department of Biology, University of Copenhagen, Helsingør, Denmark*
[2] *Department of Biology and Nordic Center for Earth Evolution (NordCEE), University of Southern Denmark, Odense, Denmark*
[3]*Department of Biochemistry and Molecular Biology and Nordic Center for Earth Evolution (NordCEE), University of Southern Denmark, Odense, Denmark*

Chromatium okenii is a large-celled highly-motile purple sulfur bacterium which is abundant at the top of the anoxic, sulfide-rich portion of the water column in meromictic Lake Cadagno in the Swiss Alps. We have exploited the tendency of this bacterium to swim to the bottom of containers to physically enrich *Chr. okenii* from 20 L of water from the chemocline. The absence of significant amounts of contaminating bacteria was confirmed by making a clone library using PCR primers for bacterial 16S rRNA. All the obtained sequences matched that reported for *Chr. okenii*. Genomic DNA from the enriched bacteria was sequenced using PacBio RS and assembled into contiguous sequences. The majority of the expected ribosomal proteins were found, supporting the idea that most of the complete genome was covered. We will report a comparison of the genes encoding components of the photosynthetic apparatus with the corresponding genes from other purple sulfur bacteria.

P22

New insights into energy conservation mechanisms in the sulfate respiring archaeon *Archaeoglobus fulgidus* VC16

William Hocking, Irene Roalkvam, Runar Stokke, Ida Helene Steen

Center for Geobiology, University of Bergen, Norway

In this study a model of the energy metabolism of the sulfate-reducing archaeon *Archaeoglobus fulgidus* is presented. The model is based on comparative transcriptome profiling of heterotrophic, hydrogenotrophic and carboxidotrophic growth utilizing either sulfate or thiosulfate as electron acceptors. The model suggests a putative lactate dehydrogenase complex (LDHs; *lldD*, *dld*, *lldEFG*), also present in sulfate-reducing bacteria, to specifically link lactate oxidation with APS reduction via the Qmo complex. Notably these LDHs were also induced during carboxydotrophic growth. High transcriptional levels of Fqo confirm an important role of $F_{420}H_2$, as well as a menaquinone-mediated electron transport chain, during heterotrophic and carboxydotrophic growth when either sulfate or thiosulfate is available. During hydrogenotrophic growth, energy conservation is probably facilitated via menaquinone to multiple membrane-bound heterodisulfide reductase (Hdr) complexes and the DsrC protein—linking periplasmic hydrogenase (Vht) to the cytoplasmic reduction of sulfite. A cytoplasmic Mvh:Hdl hydrogenase catalyzing putative bifurcation reaction seems crucial for providing the Fd_{red} needed for CO_2-fixation which thus inhibits the utilization of Fd_{red} for energy conservation. During growth on CO, when sulfate is supplied as electron acceptor, transcripts of a nitrate reductase-associated respiratory complex was induced. This complex may play a role in the integration of reduced Fd into the APS coupled respiratory chain. Genes of a singular bi-functional carbon monoxide dehydrogenase (*cdhAB-2*) were continuously highly expressed, indicating a ubiquitous role in the metabolism of CO. Finally, a putative periplasmic thiosulfate reductase was identified by specific up-regulation. Altogether, this study has identified new redox complexes and identified putative electron flow pathways specific for the utilization of different substrates and terminal electron acceptors in *A. fulgidus*. Growth on CO seems an intrinsic capability of *A. fulgidus* as dissimilatory sulfate and thiosulfate reduction functions at high partial pressures of CO with little need for induction of novel resistance mechanisms.

P23

Plant sulfate metabolism: how to economise resources under sulphate stress

Rainer Hoefgen

Max-Planck-Institute of Molecular Plant Physiology, Germany

Plants are dependent on the uptake of sulphur for growth and development. Sulfur assimilation is a tightly regulated process. Sulphate deplete conditions lead to growth retardation and yield depressions and specific responses including nutrient depletion induced senescence. The plant tries though first to mobilize resources by catabolic but also by regulatory processes which prevent flux to secondary plant metabolites, thus compromising plant defence, in favour of primary metabolism. We used combined transcriptomics and metabolomics analyses to identify processes and involved genes.

P24

Physiological and biochemical examination of the moderately thermophilic chemolithoautotroph *Thermithiobacillus* sp. ParkerM

Lee Hutt, Rich Boden

University of Plymouth, UK

The genus *Thermithiobacillus* (Wood & Kelly 2000) comprises one species with a validly published name, *Ttb. tepidarius* (DSM 3134^T, Wood & Kelly 1985), isolated from the Roman Baths at Bath (United Kingdom). The genus is one of two genera of the Class Acidithiobacillia and is poorly understood and seemingly rare – having only two strains in Culture Collections and no sequence data from molecular ecological studies. *Thermithiobacillus* sp. ParkerM (NCIMB 8349) was isolated from the sewers of Melbourne, Australia (Parker 1947) and originally identified as *Thiobacillus thioparus*. It was re-examined recently and found to be a *Thermithiobacillus* sp. (Boden et al. 2011). Chemolithoautotrophic growth of this strain was examined and compared with *Ttb. thioparus*. In batch culture, thiosulfate was oxidised stoichiometrically to tetrathionate, via a small intermediary amount of trithionate. The strain was shown to have significantly larger specific growth rate and yield (0.153 h^{-1} and 5.4 g dry biomass per mol thiosulfate) when compared to the type strain (0.047 h^{-1} and 2.8 g dry biomass per mol thiosulfate). When grown in a thiosulfate, tetrathionate or trithionate limited chemostats, it showed very similar maximum specific growth yield (Y_{max}) and maximum specific growth rate (μ_{max}) to those of *Ttb. tepidarius* for all three sulfur oxyanions. The higher yield and growth rate observed in batch culture could be owing to an increased acid-tolerance compared to the type species, allowing it to continue growth to a lower pH. Thiosulfate dehydrogenase (EC 1.8.2.2) activity was detected in cell-free extracts prepared from cells obtained from thiosulfate, tetrathionate or trithionate-limited chemostats but was significantly higher in extracts prepared from thiosulfate grown cells. The enzyme was coupled to cytochrome c_{552} in both strains of *Thermithiobacillus*, in common with other members of the Acidithiobacillia.

P25

More than a variation of a there - the structure of the electron transfer complex between the SorT sulfite dehydrogenase and its natural electron acceptor

Aaron McGrath[1], Megan Maher[2], Ulrike Kappler[3]

[1] Structural Biology Program, Centenary Institute, Locked Bag 6, NSW 2042, Australia
[2] School of Molecular Sciences, LaTrobe University, Melbourne, Vic 3086, Australia
[3] School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Qld 4072, Australia

Enzymatic sulfite oxidation is a key process in detoxification or energy generating reactions in most living cells, however, the enzymes involved in these processes differ markedly in their subunit and redox cofactor content. We have solved the structure of the first heme-free bacterial sulfite oxidizing enzyme (SOE), SorT, from the sulfonate-degrading soil bacterium Sinorhizobium meliloti. The monomers of the homodimeric SorT enzyme show the typical combination of a Molybdenum cofactor (MoCo) and a dimerization domain. However, unlike in other SOEs, the 'dimerization domain' is not actually involved in the dimerization of the enzyme, as the monomers are rotated into an 'upside down' configuration. This is a completely novel conformation for this type of enzyme which appears to be the most common type of bacterial SOE and catalytic implications are at present unclear.

The complex of SorT and its electron acceptor, SorU revealed close contact between the redox cofactors of the two proteins (Mo-heme 8.2 Å) as well as some distortion of the SorU protein on binding which may modulate the SorU redox potential and thus have significance for the reaction mechanism. Compared to the permanent complex between the SorA and SorB subunits of a previously characterized bacterial SOE the SorT/SorU complex is less stable as evidenced by a reduced buried surface area, fewer salt bridges and hydrogen bonds. Despite this, the final position of the heme and Mo redox cofactors is highly similar to what was seen in the permanent SorAB complex despite a complete lack of structural similarity between the SorB and SorU proteins. Electron transfer from SorT to SorU was found to be slower than between SorAB and its external electron acceptor but was similar to values reported for vertebrate sulfite oxidases which also require docking of a heme domain near the Mo centre.

P26

Bioinformatic analyses indicate a novel multi-enzyme system for sulfur oxidation in prokaryotes

Tobias Koch, Christiane Dahl

Institut für Mikrobiologie & Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Meckenheimer Allee 168, D-53115 Bonn, Germany

Broad range database analysis revealed that several chemo- and phototrophic sulfur oxidizing prokaryotes lacking the Dsr pathway instead contain the gene cluster *hdrC1B1AhyphdrC2B2* encoding a heterodisulfide reductase (Hdr)-like protein complex [1]. Transcriptomic [2] and proteomic [3] analyses of *Acidithiobacillus* species support the notion that this Hdr-like complex is involved in a process functionally replacing the Dsr system in the generation of sulfite. Here, we established a core set of genes present in all sulfur oxidizers containing *hdr*-like genes. Just as the *dsr* genes, *hdr*-like genes are always linked to genes for proteins involved in cytoplasmic sulfur trafficking, i.e. TusA, DsrE and often also rhodaneses. The DsrE-related proteins fall into distinct groups depending on their genetic linkage to *dsr* or *hdr* genes [4]. Furthermore, genes for lipoate-binding proteins resembling glycine cleavage system component H and genes for several enzymes responsible for biosynthesis of liponamide-containing proteins are always located in direct vicinity of *hdr*-like genes. Notably, a putative dihydroliponamide dehydrogenase is encoded immediately adjacent to the *hdr*-like genes in sulfur oxidizing archaea like *Metallosphaera cuprina* [4]. The genomic linkage of genes for proteins involved in sulfur trafficking, Hdr-like systems, liponamide-binding proteins and in archaeal sulfur oxidizers also for proteins with the potential for NAD^+ reduction in conjunction with the established potential of HdrA for electron bifurcation [5] guided us to propose new models for Hdr-linked sulfur oxidation. These include the suggestion that part of the electrons arising from sulfane sulfur oxidation can be directly transferred to NAD^+, thereby decreasing the need for energy-requiring reverse electron flow.

1. Venceslau et al. 2014. Biochim Biophys Acta 1837, 1148
2. Quatrini et al. 2009. BMC Genomics 10, 394
3. Mangold et al. 2011. Front Microbiol 2, 17
4. Liu et al. 2014. J Biol Chem 289, 26949
5. Kaster et al. 2011. PNAS 108, 2981

P27

Oxidation of molecular hydrogen in the family Beggiatoaceae

Anne-Christin Kreutzmann[1], Marc Mußmann[1], Heide Schulz-Vogt[2]

[1] *Max-Planck-Institute for Marine Microbiology, Germany*
[2] *Leibniz Institute for Baltic Sea Research Warnemuende (IOW), Germany*

Large sulfur bacteria of the family Beggiatoaceae are well known for their ability to grow on reduced sulfur compounds and various organic substrates. Other electron donors were never reported to support growth and thus are usually not considered to be of importance for members of this family. Here, we provide evidence that molecular hydrogen is an electron donor that can contribute significantly to the metabolic versatility of the Beggiatoaceae. We identified genes putatively encoding [NiFe]-hydrogenase catalytic subunits in several distantly related members of the family. These genes belonged to four phylogenetically distinct groups, which are thought to represent hydrogenases of different metabolic functions. Most of the screened strains contained more than one type of [NiFe]-hydrogenase, suggesting that the capacity for hydrogen oxidation is not only widespread in the family but that hydrogen oxidation may also play a role under different ecophysiological conditions. In order to assess the importance of hydrogen in vivo, we studied hydrogen oxidation in the chemolithoautotrophic strain *Beggiatoa* sp. 35Flor, which was grown in oxygen-sulfide gradient media with diffusional hydrogen gradients. Microsensor profiles and rate measurements suggested that the strain oxidized hydrogen aerobically in the presence of oxygen. Under these conditions, hydrogen oxidation supplied more than one third of the total electron demand and hydrogen-supplemented cultures reached significantly higher biomasses. *Beggiatoa* sp. 35Flor oxidized hydrogen also under anoxic conditions in cultures with high sulfide fluxes. Anaerobic hydrogen oxidation was most likely coupled to sulfur respiration and thus could support the disposal of internally stored sulfur to prevent physical damage resulting from excessive sulfur accumulation. Overall, molecular hydrogen could help members of the Beggiatoaceae to adapt more easily to the fluctuating conditions in microbial mats by supplying energy for maintenance and assimilatory purposes and by providing additional means to regulate the size of the internal sulfur store.

P28

The bifunctional tetrathionate reductase/thiosulfate dehydrogenase TsdA: Properties and functions of the enzymes from *Campylobacter jejuni* and *Allochromatium vinosum*

Julia Kurth[1], Kevin Denkmann[1], Sam Rowe[2], Myles Cheesman[2], Julea Butt[2], Christiane Dahl[1]

[1] Institut für Mikrobiologie & Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Meckenheimer Allee 168, D-53115 Bonn, Germany
[2] Centre for Molecular and Structural Biochemistry, School of Chemistry and School of Biological Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

The diheme *c* type cytochrome TsdA represents a novel class of bifunctional bacterial tetrathionate reductases/thiosulfate dehydrogenases. In *Allochromatium vinosum* TsdA mainly acts as a thiosulfate dehydrogenase [1] whereas the enzyme from *Campylobacter jejuni* preferentially catalyses tetrathionate reduction [2]. Both proteins contain an active site heme with rare axial His/Cys iron coordination (Heme 1). A conserved methionine is important for binding of the second heme iron (Heme 2). For CjTsdA these ligands were verified by nIR-MCD. We intend to elucidate the mechanism(s) that govern the catalytic bias of TsdA. As a first step, we used electrochemical approaches to determine the standard reduction potential of the tetrathionate/thiosulfate couple as 0.19±0.02 V and to show that CjTsdA hemes 1 and 2 are redox active in the range -450 to -270 mV and -20 to 190 mV, respectively. Regarding AvTsdA, Heme 2 has a slightly less positive and Heme 1 a much more positive redox potential than in CjTsdA. Spectroelectrochemistry revealed a redox-driven change in the axial ligands of at least Heme 2 for both proteins. Moreover, we performed detailed enzyme kinetic studies and protein film voltammetry on CjTsdA and derivatives thereof carrying replacements of the sixth axial ligands of Heme 1 and 2. Wildtype TsdA exhibited much higher substrate affinity for tetrathionate (19±1.7 µM) than for thiosulfate (440±18 µM). When thiolate ligation of Heme 1 was removed by $Cys_{138}Gly$ substitution, V_{max} decreased dramatically but substrate affinities remained virtually unchanged. Although the TsdA active site is at Heme 1, changes in ligation of Heme 2 affected substrate affinity. In summary, these findings indicate cooperativity between the two hemes and show that Heme 2 plays a pivotal role in defining the catalytic direction of this enzyme.

[1] Denkmann et al. 2012 Environ Microbiol 14, 2673.

[2] Liu et al. 2013 Mol Microbiol 88, 173

P29

The *Candidatus* "Thiodictyon syntrophicum" strain Cad16^T genome revised

Samuel Luedin[1, 2, 3], Francesco Danza[1, 2], Pierre Schneeberger[3, 4, 5], Matthias Wittwer[3], Mauro Tonolla[1, 2]

[1] University of Geneva, Sciences III, Department of Botany and Plant Biology, Microbiology Unit, 30 quai Ernest-Ansermet, CH-1211 Geneva 4, Switzerland
[2] Laboratory of applied microbiology (LAM), Department of environment, constructions and design University of applied sciences of Southern Switzerland – SUPSI, Via Mirasole 22A, CH-6500 Bellinzona, Switzerland
[3] Biology Division, Spiez Laboratory, Federal Office for Civil Protection, Austrasse, CH-3700 Spiez, Switzerland
[4] Swiss Tropical and Public Health Institute, Department of Epidemiology and Public Health, CH-4051 Basel, Switzerland
[5] Agroscope Changins-Wädenswil ACW, Department of Epidemiology and Molecular Diagnostics, CH-8820 Wädenswil, Switzerland

The meromictic alpine lake Cadagno (Switzerland) is a model ecosystem for the study of phototrophic sulfur bacteria (PSB) communities since it provides a stable vertical physiochemical gradient over time. High concentration of sulfate favors the development of microbial populations directly involved in the sulfur cycle. Members of the family *Chromatiaceae* such as *Chromatium okenii*, *Lamprocystis purpurea* and the genus *Thyocystis* and *Thiodictyon* have been extensively studied by 16S rRNA sequence analysis and have been isolated.

The CO_2 fixation capacity of *Candidatus* "Thiodictyon syntrophicum" has been studied *in vivo* and *in vitro*. Despite "T. syntrophicum" only represents 2% of the total PSB community, it provides an estimate of 25% of the total primary production in the chemocline. Further studies on the "T. syntrophicum" sulphur metabolism have led to the discovery of novel carotene ketolases and the autotrophic dicarboxylate/4-hydroxybutyrate cycle, normally found in archaea.

As a basis for further proteogenomic studies of the sulfur metabolism we sequenced the "T. syntrophicum" 7.3 Mb genome. Here we describe the features of "T. syntrophicum", in combination with the draft genome and a preliminary annotation. We thereby especially focused on the enzymatic pathways involved in sulfur metabolism and trafficking. For the *de novo* assembly and finishing of the "T. syntrophicum" genome we used a hybrid approach combining shorter IonTorrent PGM and MiSeq reads with Single Molecule, Real-Time (SMRT) PacBio reads. To complete the genome assembly and scaffolding the MIRA assembler was used together with an in-house developed software pipeline. We compared this approach with a PacBio-only assembly using HGAP.

P31

Nitrate reduction in the sulfate-reducing bacterium *Desulfovibrio desulfuricans* 27774

Angeliki Marietou[1, 2], Lesley Griffiths[1], Jeff Cole[1]

[1] *School of Biosciences, University of Birmingham, Edgbaston, Birmingham, UK*
[2] *Center for Geomicrobiology, Department of Bioscience, Aarhus University, DK-8000 Aarhus C, Denmark*

Some sulfate reducing bacteria have the ability to use nitrate as alternative terminal electron acceptor to sulfate. *Desulfovibrio desulfuricans* strain 27774 genome includes a six gene operon encoding for components of the periplasmic nitrate reductase system (*nap*). A combination of physiological and molecular approaches was used to investigate the regulation of nitrate reduction in a sulfate reducing bacterium. We report that the expression of the *nap* operon is repressed in the absence but induced in the presence of nitrate, suggesting the existence of a regulatory system in *D. desulfuricans*. Strain 27774 grew more rapidly and to higher yields of biomass with nitrate than with sulfate or nitrite as the only electron acceptor. In the presence of both sulfate and nitrate, sulfate was used preferentially, when cultures were continuously gassed to prevent sulfide inhibition of nitrate reduction. qPCR experiments confirmed that nap operon expression is regulated at the level of mRNA transcription by at least two mechanisms: nitrate induction and sulfate repression.

P32

Some studies of the function of the components of thiosulfate oxidizing multi-enzyme system from the green sulfur bacterium *Chlorobaculum tepidum*

Hidehiro Sakurai[1], Masaharu Kitashima[2], Takaaki Ito[2, 3], Kazuhito Inoue[1, 2]

[1] *Research Inst. for Photobiological H_2 production, Kanagawa University, Japan*
[2] *Department of Biological Sciences, Faculty of Science, Kanagawa University, Japan*
[3] *Medical Mycology Research Center, Chiba University, Japan (present address)*

Thiosulfate oxidation is catalyzed by the collaboration of the three proteins (SoxAXK, SoxB, and SoxYZ: the core TOMES)[1,2], and the presence of the fourth component the flavoprotein SoxF (CT1015) stimulates the reaction in the reconstituted system[3)] from the phototrophic green sulfur bacterium *Chlorobaculum tepidum*. We will discuss some details of the effects of SoxF on the core TOMES reaction including the inhibition of sulfite oxidation by SoxF. SoxYZ tends to be inactivated on storage in solution, which is ascribed in part to the oxidation of the SH group on SoxY essential to the function. The inactivated SoxY from the heterotrophic bacterium *Paracoccus pantotrophus* was reported to be reactivated greatly by incubation with sulfide but only partially with reductants such as DTT or tris(2-carboxyethyl)phosphine (TCEP) (Quentmeier et al. 2007). The inactivation and the reactivation are explained by the oxidation state of the SH group on SoxY and also by the conformational change of SoxYZ. We studied the relationship between the core TOMES activity of various preparations of SoxYZ and the changes in the molecular mass of SoxY as isolated or after various treatments by MALDI-TOF mass spectrometry. Our results suggest that in addition to the recovery of the reduced SH group from the oxidized state, we need additional assumption in order to explain the stimulating effects of sulfide.

1) Ogawa, T. et al. (2008) J. Bacteriol. 190: 6097-6110 (2008).

2) Sakurai, H. et al. (2010) Photosyn. Res. 104: 163 (2010).

3) Ogawa T. et al. (2010) Biosci. Biotechnol. Biochem. 74: 771 (2010).

P33

Study of the respiratory Arx from the purple sulfur bacterium *H. halophila*, a versatile enzyme

Barbara SCHOEPP-COTHENET[1], Marielle BAUZAN[2], Anne-lise DUCLUZEAU[3], Florence CHASPOUL[4], Mahmoud HAJJ CHEHADE[5], Fabien PIERREL[5], Wolfgang NITSCHKE[1]

[1] Bioénergétique et Ingénierie des Protéines (UMR 7281)/CNRS, Aix Marseille University, IMM FR3479, Marseille, France
[2] Institut de Microbiologie de la Méditerranée, FR3479, F-13402 Marseille Cedex 20, France
[3] Beadle Center, University of Nebraska-Lincoln, 1901 Vine Street, Lincoln, NE 68588-0660, USA
[4] Unité de Chimie-Physique, Faculté de Pharmacie 13005, Marseille, France
[5] Chimie et Biologie des Métaux, (UMR5249), CEA/CNRS/ JFUniversity, Grenoble, France

The three presently known enzymes responsible for arsenic-using bioenergetic processes are arsenite oxidase (Aio), arsenate reductase (Arr) and alternative arsenite oxidase (Arx), all of which are molybdoenzymes from the group referred to as the Mo/W-bisPGD enzyme superfamily. Since arsenite is present in substantial amounts in hydrothermal environments (frequently considered as vestiges of primordial biochemistry), arsenite-based bioenergetics has early on been predicted to be ancient. Conflicting scenarios, however, have been put forward proposing either Arr/Arx or Aio as operating in the ancestral metabolism. Phylogenetic data argue in favor of Aio whereas biochemical and physiological data led several authors to propose the Arx/Arr enzyme as the most ancient anaerobic arsenite oxidising enzyme. Here we combine phylogenetic approaches with physiological and biochemical experiments, studying the Arx from the purple sulfur bacterium *Halorhodospira halophila*. This strain can use As(III) instead of sulfide as electron donor to sustain photosynthetic growth. We show that under physiological conditions, Arx reacts with ubiquinone to oxidise arsenite, in line with thermodynamic considerations. Under non physiological conditions, we can, however, force Arx to reacts in reverse, with menaquinone to reduce arsenate. The phylogeny of the quinone biosynthesis pathway, clearly indicates that the ubiquinone pathway is recent. An updated phylogeny of Arr/Arx furthermore indicates a recent emergence of this enzyme. We therefore conclude that the As(III) oxidation metabolism involving Arx is recent and that only the metabolism involving Aio can have performed anaerobic As(III) oxidation in the Archaean. Phylogeny moreover revealed close phylogenetic proximity between Arr/Arx and Psr or Ttr, two enzymes involved in the respiration of sulfur compounds. The evolutionary adaptations linking Arr/Arx and Ttr/Psr are therefore expected to mainly involve tinkering with substrate affinities in the catalytic site. With the aim to establish the structural basis of this tinkering, we initiated the study of Arx reactivity towards sulfur compounds.

P34

Exploring the currently unknown pathway of elemental sulfur disproportionation by comparative transcriptomics with *Desulfurivibrio alkaliphilus*

Lars Schreiber[1], Casper Thorup[2], Kai Finster[2]

[1] *Department of Bioscience, Center for Geomicrobiology, Aarhus University, Denmark*
[2] *Department of Bioscience, Section of Microbiology, Aarhus University, Denmark*

The microbially mediated disproportionation of elemental sulfur represents the energy-yielding transformation of elemental sulfur to sulfate and sulfide. Significant numbers of bacteria closely related to known disproportionaters are detected in marine environments, aquifers, and corrosive oil pipeline biofilms which indicates that sulfur disproportionation may play an important role in many habitats. The exact importance and ubiquity of sulfur disproportionation, however, is currently difficult to determine as (1) its end products are quickly removed by sulfide-oxidizing microorganisms or sulfate-reducer, and (2) marker genes that could help to determine the presence and activity of sulfur disproportionators are currently unknown. This study targets the latter problem and tries to elucidate the genetic basis of the disproportionation pathway by using transcriptomics. We will present first results of gene expression patterns of *Desulfurivibrio alkaliphilus* grown under nitrate-reducing and sulfur disproportionation conditions.

P35

Desulfurivibrio alkaliphilus AHT2 can couple the nitrate dependent oxidation of sulphide to growth

Casper Thorup[1], Lars Schreiber[2], Kai Finster[1]

[1] *Department of Bioscience, Aarhus University, Aarhus, Denmark*
[2] *Center for Geomicrobiology, Aarhus University, Aarhus, Denmark*

Sulphide oxidation has earlier been documented for sulphate reducers in which sulphide is oxidized to sulphate using nitrate as electron acceptor, but growth could not be documented. In this study we provide one of the rare examples that shows that a member of the delta proteobacteria and close relative to *Desulfobulbus*, namely the haloakalophilic strain *Desulfurivibrio alkaliphilus* AHT2, can couple the oxidation of sulphide and reduction of nitrate to ammonium to growth. Triplicate cultures were grown in 100 mL screw-capped bottles containing a modified *Dethiobacter* medium with the addition of 3 mM nitrate and incubated at 30°C. Samples were taken at eight hour intervals over two days. Nitrate and sulphate concentrations were determined by ion-chromatography and sulphide and ammonium concentrations were determined using spectrophotometric methods. Cell numbers were obtained by fluorescence microscopy upon staining with SyBr gold. The consumption of sulphide and nitrate is accompanied by the concomitant production of ammonium and sulphate. The net change in concentration of substrates and products can be summarized by the following stoichiometry: $H_2S + NO_3^- + H_2O \rightarrow SO_4^{2-} + NH_4^+$. This signifies a net flow of eight electrons from sulfur to the nitrogen. An increase in cell density was observed. The results clearly demonstrate anaerobic growth in *Desulfurivibrio alkaliphilus* AHT2 by sulphide oxidation. The next step is to elucidate the pathway of sulphide oxidation in strain *Desulfurivibrio alkaliphilus* AHT2 and to uncover why only few delta-proteobacteria can couple this process to growth.

P36

Isolation of novel acidophilic sulfate-reducing bacteria for bioremediation of acid rock drainage

Irene Sánchez-Andrea[1], D. Barrie Johnson[2], Alfons J. M. Stams[1]

[1] Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands
[2] School of Biological Sciences, University of Wales, Bangor LL57 2UW, UK

Acid rock drainage (ARD) refers to acid and metalliferous waters produced by the biochemical oxidation of metallic sulfide-ores (e.g. pyrite). It produces long-term contamination of aquifers, with secondary effects like reduced crop yields, death of fish, etc. The environmental concern has led to increasing efforts to remediate ARD environments and biological treatment applying sulfate-reducing bacteria (SRB) has become a very attractive option. SRB produce alkalinity, neutralizing the AMD; and sulfide, which reacts with chalcophilic metals in solution and precipitates them as insoluble metal sulfides. The use of acidophilic SRB may become crucial for the process; it avoids the need of influent neutralization and it allows a selective metal recovery by controlling the pH of the bioremediation reactor. While there is widespread evidence of sulfate reduction in natural and anthropogenic low pH environments, most SRB obtained from these sites do not grow below pH 5, and there have been relatively few reports of acidophilic/acid-tolerant isolates. Due to their biotechnological importance, we have made an exhaustive effort to enrich and isolate novel acidophilic SRB. Two novel species within the *Desulfosporosinus* genus have been obtained, one recently published as *D. acididurans*. Another strain represents a new genus in the same *Peptococcaceae* family, proposed as a *Desulfobacillus* sp. The strains showed promising characteristics for their application such as broad range of growth in terms of pH (from pH 3.8 to pH 7) and temperature (from 15°C to 42°C); high metal tolerance (up to 50 mM ferrous iron and 10 mM aluminium) and broad spectrum of substrate utilization for sulfate reduction (sugars, alcohols, organic acids and hydrogen). Enrichments and novel strains have been tested in bioreactors fed with synthetic acid mine drainage waters containing different mixtures of heavy metals. The experiments showed the ability of the microorganisms to perform sulfidogenesis on those extreme conditions and their potential for selective metal recovery.

P37

Molecular basis of sulfur metabolism in *Rhodococcus* sp. AF21875, a strain that prefers organosulfur compounds over sulfate

Valeria Tatangelo[1], Jonathan Van Hamme[2], Eric Bottos[2], Giuseppina Bestetti[1], Andrea Franzetti[1]

[1] *Department of Earth and Environmental Sciences, University of Milano-Bicocca, Italy*
[2] *Department of Biological Sciences, Thompson Rivers University, Canada*

Sulfur is an essential element for bacterial growth and, when bacteria are under sulfate-limiting conditions, they can utilize alternative sulfur sources such as aliphatic sulfonates. The uptake and utilization of aliphatic sulfonates and taurine are controlled by the *ssu* and *tau* systems, respectively. In both systems an ABC transport system (*ssuABC* and *tauABC*) and an oxygenase system (*ssuDE* and *tauD*) are present. Several mycolata can utilize dibenzothiophene (DBT) and its derivatives as sulfur source using systems such as the *dsz* and DOX operons. Rhodococci are well known for their ability to catabolize organic pollutants and have previously been found to be capable of exploiting a wide range of organosulfur compounds for sulfur requirements. We have isolated *Rhodococcus* species strain AF21875, an organism that uses dibenzothiphene over sulfate as a preferred sulfur source, and generates 2-hydroxybiphenyl as a byproduct indicating that it is using the DBT desulfurization pathway encoded by the dsz operon. Here we present a whole genome shotgun of AF21875 and describe a wide variety of genes associated with organosulfur transport and assimilation that were identified during annotation. In the AF21875 genome, both the assimilatory sulfate reduction pathway and the cysteine biosynthesis pathway have been detected. In addition, *tauABCD* and *ssuEADCB* genes were annotated, along with a putative plasmid harboring the *dszABC* genes. Normally sulfate inhibits organosulfur metabolism, so we are investigating the molecular features in AF21875 resulting in the unique phenotype observed as there exists significant potential for industrial application.

P38

Biochemical and functional analysis of sulfide oxidase flavoproteins from purple sulfur bacterium, *Thiocapsa roseopersicina*

András Tóth[1, 2], Ágnes Duzs[2], Tímea Balogh[2], Brigitta Németh[1], Enikő Kiss[1], Gábor Rákhely[1, 2]

[1] *Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, H-6726 Szeged, Hungary*
[2] *Department of Biotechnology, University of Szeged, H-6726 Szeged, Hungary*

Sulfide oxidase flavocytochrome *c* (Fcc) and sulfide quinone oxidoreductase (Sqr) enzymes are ancient flavoproteins, widely present in different domains of life. These enzymes can involve in energy metabolism of microorganisms, regulation of cellular sulfide concentration in eukaryotic cells or protection against toxic sulfide. The phototrophic purple sulfur bacterium *Thiocapsa roseopersicina* has a versatile sulfur metabolism. Three genes presumably encoding flavocytochrome *c* (*fcc*) and two different sulfide quinone oxidoreductase type enzymes (*sqrD*, *sqrF*) were identified in the genome sequence. Phylogenetic and comparative sequence analysis of these proteins revealed that SqrD and SqrF belong to partially characterized groups (type IV and VI) of Sqr enzymes. For identification of function of these proteins Δ*fcc* and Δ*sqrF T. roseopersicina* strains were created and analysed. The Fcc mutant strain completely lost the periplasmic sulfide oxidase activity. SqrF mutant cells possessed slightly decreased sulfide consumption rate. The effect of sulfide on the expression of the identified genes was studied by qRT-PCR. This analysis revealed that expression of the flavocytochrome *c* and the sulfide quinone oxidoreductases are activated at different sulfide concentrations. Fcc and Sqn proteins fused to Strep II tag were expressed in *T. roseopersicina* and purified. The reduction and oxidation of the pure recombinant proteins in the presence of sulfide and appropriate electron acceptor were followed by UV-Vis spectra. The Fcc and SqrF proteins catalyzed the expected sulfide dependent cytochrome *c* or quinone reduction reactions, respectively. Kinetic analysis revealed that affinity of Fcc and SqrF to sulfide is considerably different which suggests in well correlation with the expression studies that these enzymes have role in the metabolism of cells at different sulfide conditions.

P39

Comparative proteomics reveals two methanol-degrading pathways in the sulfate reducing bacterium *Desulfotomaculum kuznetsovii*

Michael Visser, et al.

Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands

Several phylogenetic groups of microorganisms are able to grow with methanol as a sole carbon and energy source. Aerobic and facultative anaerobic methylotrophs generally oxidize methanol to formaldehyde by using a methanol dehydrogenase, while anaerobic methylotrophs such as methanogens and acetogens are known to use a methanol methyltransferase system. However, the methanol metabolism of sulfate-reducing bacteria has not been extensively studied. Previous work with the sulfate-reducing bacterium *Desulfotomaculum kuznetsovii* resulted in a partially purified alcohol dehydrogenase that showed activity with methanol and ethanol. However, the genome also indicated the presence of a methanol methyltransferase system. Therefore, the methanol metabolism in *D. kuznetsovii* remained unsolved. The methanol methyltransferase system is vitamin B12 dependent. Therefore, we studied the methanol metabolism of *D. kuznetsovii* by growing the cells with methanol and sulfate in the presence and absence of cobalt and vitamin B12. When cobalt and vitamin B12 were omitted from the medium *D. kuznetsovii* showed a decreased rate of methanol conversion. A subsequent comparative proteomics approach, using nanoLC-MS/MS, helped to unravel the methanol metabolism of *D. kuznetsovii*. Cells were grown under four different conditions: Methanol and sulfate in presence and absence of cobalt and vitamin B12, lactate and sulfate, and ethanol and sulfate. The lactate growth condition was used as a reference. Proteomic results indicate the presence of two methanol degrading pathways in *D. kuznetsovii*, a cobalt dependent methanol methyltransferase system and a cobalt independent alcohol dehydrogenase. The alcohol dehydrogenase is also involved in the ethanol metabolism of *D. kuznetsovii*. This study is the first evidence for the occurrence of two methanol degrading systems in a bacterium.

P40

Revealing the genotypic and phylogenetic diversity within the genus *Thioalkalivibrio*

Anne-Catherine Ahn[1], Lex Overmars[1], Michael Richter[2], Jan P. Meier-Kolthoff[3], Judith Umbach[4], Dimitry Y. Sorokin[5, 6], Gerard Muyzer[1]

[1] Microbial Systems Ecology, Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands
[2] Ribocon, Bremen, Germany
[3] Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
[4] Microbial Genomics and Bioinformatics, Max-Planck-Institute for Marine Microbiology, Bremen, Germany
[5] Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia
[6] Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

Members of the genus *Thioalkalivibrio* are obligate chemolithoautotrophic and haloalkaliphilic sulfur-oxidizing bacteria. They live in the dual extreme environment of soda lakes that have a pH ranging from 9.5 to 11 and a salt concentration up to saturation. A previous study revealed a high genetic diversity through rep-PCR fingerprint analysis and furthermore suggested an endemic character for members of this genus. Having now the genome sequences of 77 *Thioalkalivibrio* strains isolated from soda lakes from various locations all over the world, different phylogenetic and genotypic approaches, such as 16S rRNA, supertree analysis of orthologous protein sequences, ANI (Average Nucleotide Identity) and in silico DDH (DNA-DNA hybridization) were applied in order to define the diversity within the genus. With these analyses, the high genetic diversity could be confirmed and the affiliation of the different strains to the described species could be determined in detail. Based on this approach, we found 14 new genetic species next to already 10 described species in this genus. Furthermore, the results show that the monophyletic character of this genus may be questionable, as strains from other genera were found within the branch of the genus *Thioalkalivibrio* in phylogenetic trees. As 3 species were separated from the rest of *Thioalkalivibrio*, which are still grouped around their type species *Tv. versutus*, these outliers should be redefined within a novel genus.

P41

Genomics and proteomics of *Thioploca ingrica*, a nitrate-storing sulfur oxidizer living in freshwater sediments

Hisaya Kojima, Tomohiro Watanabe, Manabu Fukui

The Institute of Low Temperature Science, Hokkaido University, Japan

Sulfur-oxidizing bacteria which accumulate a high concentration of nitrate are important constituents of aquatic sediment ecosystems. For these microorganisms, only fragmented draft genome sequences have been available. In this group, *Thioploca ingrica* is the sole species thriving in freshwater environments at present. By performing metagenomic analysis, the complete genome of *Thioploca ingrica* was successfully reconstituted. Sulfur oxidation pathway deduced from the reconstituted genome was identical to that suggested for the closest relatives of *Thioploca*. In the genome, a full set of genes for nitrate reduction to dinitrogen gas was identified. Further, a proteomic analysis was performed to investigate the physiology of *Thioploca* in lake sediments. In the analysis, many proteins involved in sulfur oxidation, nitrate respiration, and inorganic carbon fixation were detected as major components of the protein extracts.

Kojima,H., Ogura,Y., Yamamoto,N., Togashi,T., Mori,H., Watanabe,T., Nemoto,F., Kurokawa,K., Hayashi,T., and Fukui,M. Ecophysiology of Thioploca ingrica as revealed by the complete genome sequence supplemented with proteomic evidence. The ISME Journal : in press.

P42

Diversity and abundance of sulfate-reducing alkane degraders in cold marine surficial sediments – microbial targets in oil and gas exploration

Antje Gittel, Johanna Donhauser, Kasper Kjeldsen, Hans Røy, Bo Barker Jørgensen

Center for Geomicrobiology, Department of Bioscience, Aarhus University, Aarhus, Denmark

The understanding that hydrocarbons migrate from marine subsurface petroleum reservoirs to the sediment surface recently led to the usage of gas seep detection as an oil prospecting tool. Besides remote sensing and analysis of seep gas composition, it has been hypothesized that the elevated abundance of microbial targets, in particular sulfate-reducing short-chain alkane (SCA) degraders, might indicate the presence of seeps and thus petroleum reservoirs. However, the diversity of these microorganisms in cold marine surficial sediments is largely unexplored and probably underestimated. In addition, the origin and turnover of short-chain hydrocarbons in these sediments has not been assessed so far.

A functional gene approach targeting a subunit of the 1-methylalkyl succinate synthase (*masD*) gene was combined with single cell genomics and gas analytics to (1) assess the diversity and abundance of sulfate-reducing SCA degraders in surficial sediments with and without seepage, (2) unravel novel diversity and (3) determine the potential for bioprospecting assays using elevated *masD* gene abundance as a seepage indicator.

Database research and a pilot study on potential oil exploration sites in the North Sea revealed that existing *masD* gene primer pairs did not comprehensively target the known *masD* gene diversity. We thus developed an improved detection assay and successfully applied it to sediment samples from seepage and non-seepage sites. *masD* genes were found in all these samples, showing that putative SCA degraders were present both at seepage-impacted sites and reference sites that are currently not impacted by hydrocarbon seepage. Their ubiquitous presence indicated that, in the event of seepage, SCA degraders might grow to represent a larger fraction of the community and will be readily detectable in bioprospecting assays. However, further data analysis is needed to assess distinct seep-specific diversity and to link increased gas concentrations to elevated abundances of the respective target genes.

P43

Exploring the pan-genome of the haloalkaliphilic sulfur-oxidizing bacteria of the genus *Thioalkalivibrio*

Lex Overmars[1], Dimitry Y. Sorokin[2, 3], Gerard Muyzer[1]

[1] *Microbial Systems Ecology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands*
[2] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*
[3] *Department of Biotechnology, Delft University of Technology, Delft, The Netherlands*

Thioalkalivibrio is a genus of chemolithoautothrophic sulfur-oxidizing bacteria capable of growing at pH up to 10.5 and sodium concentrations up to 4.3 M. An insight into their genomic potential and diversity will help us to better understand their core metabolism and the molecular mechanisms by which *Thioalkalivibrio* have adapted to these extreme conditions.

As part of the community sequence program of the Joint Genome Institute (JGI) the genomes of 70 *Thioalkalivibrio* strains were sequenced. These strains were isolated from soda lakes in Mongolia, Siberia, California, Egypt and Kenya, which vary in salinity and chemistry. Comparative analysis revealed that the pan-genome consists of more than 10,000 orthologous groups (OGs), of which ~15% make up the conserved core. The core genome is mainly composed of housekeeping, (core-) metabolism- and information storage/processing-related genes, whereas the accessory genome is characterized by an overabundance of genes involved in signal transduction and cell wall biogenesis. We have clustered the strains based on presence/absence of OGs and found two major clusters consisting of isolates from the Asiatic (Siberia and Mongolia) and the African (Kenya and Egypt) continents, respectively. Interestingly, we observed a relative high variability in presence of genes involved in the sulfur metabolism among the 70 strains. We are currently extending our comparative genome analysis with other pathways and proteins potentially important for the adaptation to their environment to learn more about how *Thioalkalivibrio* can flourish at these conditions of high pH and salinity.

P44

Enabling large-scale ecological studies of sulfate/sulfite-reducing microorganisms: A new approach for highly parallel Illumina sequencing of *dsrA* and *dsrB* amplicons

Claus Pelikan, Craig Herbold, Alexander Loy

Division of Microbial Ecology, Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna, Vienna, Austria

Microorganisms that anaerobically respire sulfate, sulfite or organosulfonates contribute significantly to the world's sulfur and carbon cycles. The central step of these processes is the reduction of sulfite to hydrogen sulfide, which is catalyzed by a reductive version of the highly conserved enzyme dissimilatory (bi)sulfite reductase (DsrAB). Several *dsrAB*-targeted primers are available and commonly applied for amplicon based community analyses, however next generation sequencing (NGS) library preparation methods and data analysis tools remain underevaluated for processing of *dsrAB* amplicon sequences. Furthermore, most published primers for *dsrAB* that are suitable for NGS library preparation have a rather limited coverage. Here, we describe an Illumina sequence preparation and analysis pipeline for *dsrA* and *dsrB* gene fragments. These were amplified with a new, highly flexible two-step barcoding procedure, including a first PCR with new highly degenerate *dsrA* and *dsrB* primers and a second PCR with universal barcoding primers. The updated and newly developed *dsrA* and *dsrB*-targted primer sets covered between 97-100% of the known diversity of bacterial-type, reductive *dsrAB* and produced NGS-compatible amplicons of about 300 bp and 700 bp in size, respectively. Amplicon preparation, data quality, and analysis procedures were initially evaluated with two complex mock communities of defined diversity and subsequently applied to selected environmental samples of unknown composition. Although single nucleotide primer mismatches and other PCR biases had notable effects on data structure, most sequences from the mock communities were retrieved in relative abundances proportional to input relative abundances. The new primer sets and optimized sequence analysis pipeline now enables large-scale diversity analyses of bacterial-type, reductive *dsrAB*-carrying microorganisms in the environment or under controlled experimental conditions.

P45

Disproportionation of sulfur compounds by thermophilic microorganisms

Alexander Slobodkin

Winogradsky Institute of Microbiology, Russian Academy of Sciences, Russia

Microorganisms that disproportionate sulfur compounds such as thiosulfate, sulfite or elemental sulfur are a unique group of sulfur-cycling prokaryotes that play an important role in modern sedimentary environments and probably have been involved in sulfur transformations in early archaean ecosystems. Inorganic sulfur compounds fermentation that supports microbial growth has been reported for 27 species of 14 genera mainly in the Proteobacteria. Most of them are able to dismutate thiosulfate and sulfite, while the disproportionation of elemental sulfur is more rare physiological property. Thermodynamic calculations show that S^0 disproportionation is more energetically favorable at elevated temperatures. Elemental sulfur is abundant in thermal environments, however before our studies, the capacity to grow by disproportionation of S^0 among thermophiles was not known. Recently we have isolated and characterized two novel thermophilic elemental sulfur-disproportionating bacteria - *Thermosulfurimonas dismutans* and *Dissulfuribacter thermophilus* from deep-sea hydrothermal vents (Slobodkin et al., 2012; 2013). Both microorganisms are chemolithoautotrophs able to gain energy for growth by disproportionation of elemental sulfur, thiosulfate or sulfite and unable to perform dissimilatory sulfate reduction. *Thermosulfurimonas dismutans* represents the new genus of the phylum Thermodesulfobacteria, while *Dissulfuribacter thermophilus* forms distinct phylogenetic branch within Deltaproteobacteria. Further studies on sulfur disproportionation in continental freshwater hot springs lead us to the isolation of new microorganisms 'Dissulfurimicrobium hydrothermalis' that also belong to Deltaproteobacteria. Investigations of physiological mechanisms of low soluble S^0 disproportionation indicate the involvement of polysulfides in this process. Overall, the data on isolation of lithoautotrophic sulfur-disproportionating microorganisms from deep-sea hydrothermal vents and freshwater hot springs point to important role of this physiological group in sulfur-rich extreme environments.

1. Slobodkin et al. (2012) Int J Syst Evol Microbiol 62:2565-2571

2. Slobodkin et al. (2013) Int J Syst Evol Microbiol 63: 1967-1971

P46

Novel thermophilic bacteria capable of chemolithotrophic anaerobic sulfur oxidation

Galina Slobodkina

Winogradsky Institute of Microbiology RAS, Russia

Sulfur-oxidizing microorganisms are phylogenetically diverse and include photolithotrophic bacteria and chemolithotrophic bacteria and archaea. Thermophilic sulfur-oxidizing bacteria are known among the phyla Aquificae and Proteobacteria. Obligately anaerobic species of elemental sulfur oxidizers have not been previously reported. In present study, we report the isolation of two strains of thermophilic anaerobic chemolithotrophic bacteria capable of reduced sulfur compounds oxidation. Strain ST65 was isolated from the deep-sea hydrothermal vent (1910 m deep) on the Eastern Lau Spreading Center. Cells were straight or slightly curved short rods, 0.5 to 0.6 μm in diameter and 0.8 to 1.5 μm in length. The strain grew at 65oC and pH 6.5-6.8 under anaerobic conditions coupling elemental sulfur oxidation with nitrate reduction. Nitrate was reduced to ammonia. It was also capable of sulfur disproportionation. Organic substrates did not stimulate growth. The closest relatives of the isolated organism were *Thermoslfurimonas dismutans* and *Thermodesulfatator atlanticus* (89.8 and 89.5% of 16S rRNA gene similarity respectively). We propose to assign strain ST65 to a new species of a novel genus 'Thermosulfurisoma ammonica' in the order Thermodesulfobacteriales. Strain S2479 was isolated from the marine shallow-water hydrothermal vent (Kunashir Island, Russia). It had rod-shaped cells that grew anaerobically at 65°C and pH 6.5-6.8 with elemental sulfur or thiosulfate as electron donors and nitrate as electron acceptor. 16S rRNA gene sequence analysis revealed that the strain S2479 belongs to the order Chromatiales being equidistant from the representatives of families Ectothiorhodospiraceae, Chromatiaceae and Thioalkalispiraceae (91-92%).

P47A

Tracking carbon flow from major classes of biomolecules into microorganisms under psychrophilic sulfate-reducing conditions in Arctic marine sediments

Kenneth Wasmund[1], Claus Pelikan[1], Albert Leopold Müller[1], Julia Rosa de Rezende[2], Kasper Urup Kjeldsen[2], Bo Barker Jørgensen[2], Alexander Loy[1]

[1] *Division of Microbial Ecology, University of Vienna, Austria*
[2] *Center for Geomicrobiology, Aarhus University, Denmark*

To understand the microbial ecology and biogeochemistry of marine sediments under permanently cold conditions that prevail at approximately 90% of the seafloor, it is important to know and understand the organisms involved in the mineralisation of detrital biomass and major classes of cellular-derived macromolecules. Such macromolecules comprise most of the bioavailable organic matter and therefore nutrients and/or energy for microbial communities in marine sediments. This research therefore investigates microbial communities involved in the degradation of cellular biomass including selected macromolecules, i.e. proteins, lipids and nucleic acids, in marine sediments from Arctic sediments under cold (4°C) sulfate-reducing conditions. To reveal how different sediment microorganisms interact to degrade complex organic molecules and thus influence the flow of carbon through the anaerobic microbial food web, we performed microcosm incubations with ^{13}C-labelled cyanobacterial (*Spirulina*) biomass and individual ^{13}C-labelled macromolecules (proteins, lipids, nucleic acids) or a model fermentation product (acetate). We analysed these incubations by amplicon sequencing of bacterial 16S rRNA and dissimilatory (bi)sulfite reductase (*dsrAB*) genes from different time-points and isopycnic density gradient fractions (i.e. stable isotope probing). Data currently obtained from 16S rRNA gene sequencing clearly implicates several taxa as primary degraders of different macromolecule classes. These taxa exhibited fast responses (i.e. within 2 days) to substrate additions compared to controls, indicating a primed and active community. Some of these taxa were also observed as primary-degraders of whole-cell cyanobacterial biomass, and our data thereby provides insights into the partitioning of carbon derived from this cellular biomass. Sulfate-reducing deltaproteobacterial taxa generally displayed a slower response to macromolecule additions, suggesting they used fermentation products derived from primary degraders. Incubations with acetate as a model fermentation product also supported these findings. On-going analyses aim to further elucidate the role of different sulfate-reducers under the different experimental treatments.

P47B

Changes in Baltic Sea sediment oxygen concentrations induces changes in facultatively anaerobic sulfide oxidizing genera *Sulfurimonas* and *Sulfurovum*

Elias Broman, Johanna Sjöstedt, Jarone Pinhassi, Mark Dopson

Linnaeus University, Sweden

Eutrophication of the Baltic Sea increases algal bloom frequency and magnitude. Eventually these blooms decay and a portion of the biomass reaches the sediment. Microbial communities degrade this carbon, consuming the available oxygen, which results in sediments commonly referred to as “dead zones”. This study investigated how the microbial community structure and metabolic pathways in the surficial sediment changes as a result of transitions between oxic and anoxic conditions. A transition from oxic to anoxic conditions resulted in decreased sulfate, nitrite, and nitrate. In contrast, converting anoxic sediments to oxic conditions caused an increase in sulfate concentration. Large changes in the microbial community based upon next generation sequencing of the 16S rRNA gene were primarily related to the relative abundance of the facultatively anaerobic sulfide oxidizing genera *Sulfurimonas* and *Sulfurovum*. These genera are chemolitoautotrophic bacteria, able to oxidize zero-valence sulfur and hydrogen sulfide using either oxygen or nitrate as an electron acceptor. This study allowed identification of the changes in microbial community as a response to oxygen concentrations that were primarily associated with sulfur redox change.

P47C

Sulfide oxidation by cable bacteria: Getting by with a little help from their (associated) friends?

Signe Brokjær Nielsen[1], Steffen Larsen[1,2], Jesper Tataru Bjerg[1], Lars Schreiber[2], Kasper U. Kjeldsen[2], and Andreas Schramm[1,2]

[1] Section for Microbiology, Department of Bioscience, Aarhus University, Aarhus, Denmark
[2] Center for Geomicrobiology, Department of Bioscience, Aarhus University, Aarhus, Denmark

Cable bacteria are filamentous members of the Desulfobulbaceae that couple oxygen reduction at the sediment surface with sulfide oxidation in deeper, anoxic sediment layers by conducting electrons over centimeter distances. No direct proof exists that cable bacteria themselves oxidize sulfide: they can so far not be grown in pure culture, and their closest cultured relative is the sulfate reducer *Desulfobulbus propionicus*. We have previously found that sulfide-oxidizing bacteria (SOB) co-establish with cable bacteria in suboxic sediment layers (Schauer et al., 2012), and a recent study demonstrated that SOB fix CO_2 in sediment with intact cable bacteria but not when the current is interrupted (Vazques et al., 2015).

The aim of the current study was to describe the microbial community associated with cable bacteria in more detail. We re-investigated data from marine cable enrichments, analyzed sequences retrieved from single, manually picked and cleaned filaments from numerous locations, and sequenced autoclaved freshwater sediment inoculated with cable bacteria. Our combined data show that both marine and freshwater cable bacteria consistently associate with three main classes of SOB; the exact SOB type may differ based on the original habitat sampled. Detection of transcripts of key genes for sulfide oxidation in the suboxic zone furthermore suggests that these SOB are active even without direct contact to oxygen. We propose that the associated SOB can deliver electrons from sulfide oxidation to the cable bacteria. The mechanism for such an interspecies electron transfer, the significance of the associated SOB for electrogenic sulfide oxidation, and whether cable bacteria at all participate in sulfide oxidation, remains to be investigated.

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2. Schauer R., Larsen S., Kjeldsen U K., Bjerg T J., Schreiber L., Risgaard-Petersen N., Nielsen L.P. & Schramm A. (2012) Development and succession of electrogenic microbial communities in marine sediment. In: ISME14, Copenhagen, Denmark.

3. Vasquez-Cardenas D., Malkin S.Y., van de Vossenberg J., Polerecky L., Schauer R., Middelburg J.J., Meysman F.J.R. & Boschker H.T.S. (2014) Microbial communities and carbon metabolism associated with electrogenic sulfur oxidation in coastal sediments. In: ISME15, Seoul, South Korea.

P48

A unique isotopic fingerprint during sulfate-driven anaerobic oxidation of methane

Gilad Antler[1], Alexandra V. Turchyn[1], Barak Herut[2], Orit Sivan[3]

[1] *Department of Earth Sciences, University of Cambridge, Cambridge CB2 3EQ, UK*
[2] *Israel Oceanographic and Limnological Research, National Institute of Oceanography, Haifa 31080, Israel*
[3] *Department of Geological and Environmental Sciences, Ben Gurion University, Beer Sheva 84105, Israel*

Bacterial sulfate reduction is responsible for the majority of anaerobic methane oxidation in modern marine sediments. This sulfate-driven AOM can often metabolize all the methane produced within marine sediments, preventing any from reaching the overlying ocean. In certain areas, however, methane concentrations are high enough to form bubbles, which can reach the seafloor, only partially metabolized through sulfate-driven AOM; these areas where methane bubbles into the ocean are called cold seeps, or methane seeps. We use the sulfur and oxygen isotopes of sulfate in locations where sulfate-driven AOM is occurring both in methane seeps as well as lower flux methane transition zones to show that in methane seeps, the sulfur and the oxygen isotope data during the coupled sulfate reduction fall into a very narrow range and with a close to linear relationship (slope 0.37± 0.01—R^2= 0.98, n=52, 95% confidence interval). In the studied environments, considerably different physical properties exist, excluding the possibility that this linear relationship can be attributed to physical processes such as diffusion, advection or mixing of two end-members. This unique isotopic signature emerges during bacterial sulfate reduction by methane in 'cold' seeps and differs when sulfate is reduced by either organic matter oxidation or by a slower, diffusive flux of methane within marine sediments. We show also that this unique isotope fingerprint is preserved in the rock record in authigenic build-ups of carbonates and barite associated with methane seeps, and may serve as a powerful tool for identifying catastrophic methane release in the geological record.

P49

Thiosulfate dehydrogenase (TsdA) from *Allochromatium vinosum*: Structural and functional insights into thiosulfate oxidation

José A. Brito[1], Kevin Denkmann[2], Inês A. C. Pereira[2], Christiane Dahl[2], Margarida Archer[1]

[1] Instituto de Tecnologia Química e Biológica – António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal
[2] Institut für Mikrobiologie & Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, Germany

The ability to perform the very simple oxidation of two molecules of thiosulphate to tetrathionate is widespread among prokaryotes. Despite the pervasive occurrence of tetrathionate formation, and its well-documented significance within the sulphur cycle, little is known about the enzymes catalysing the oxidative condensation of two thiosulphate anions. To fill this gap, the thiosulphate dehydrogenase (TsdA), enzyme from *Allochromatium vinosum*, was recombinantly expressed, purified and characterized. Moreover, we solved the "as isolated" crystal structure of the enzyme and further obtained X-ray structures of TsdA in several redox states. The protein crystallized in space group C2 with PEG 3350 as precipitant and one molecule in the asymmetric unit. TsdA contains two typical class I *c*-type cytochrome domains with two hemes axially coordinated by His53/Cys96 and His164/Lys208. The X-ray structure showed an all-alpha structure with structural similarities to the *Rhodovulum sulfidophilum*'s SoxAX (PDB code 2OZ1), and the low-redox-potential cytochrom c_6 from *Hizikia fusiformis* (PDB code 2ZBO). Interestingly, reduction of the enzyme causes a Lys208/Met209 ligand switch in heme 2. TsdALys208Asn or Lys208Gly variants exhibit similar substrate affinities as the wildtype protein but much lower specific activities pointing at this heme as the electron exit point. Cys96 is essential for catalysis. Overall, our kinetic, spectroscopic and structural data lead us to propose a mechanism where two thiosulfate molecules enter the active site, inducing a movement of the Sγ of Cys96 out of the iron coordination sphere; this ligand movement results in an increase of the redox potential of heme 1, thus allowing the sequential uptake of the two electrons resulting from the conversion of the two thiosulfates to tetrathionate, leading to the reduction of both hemes; upon reduction, heme 2 undergoes a ligand switch, which increases its redox potential and hinders the back reaction.

P50

Comparative analyses of haloalkaliphilic sulfidogens from soda lakes

Emily Denise Melton[1], Kai Finster[2], Dimitry Sorokin[3, 4], Gerard Muyzer[1]

[1] *Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, the Netherlands*
[2] *Department of Bioscience, Microbiology Section, Aarhus University, Aarhus, Denmark*
[3] *Winogradsy Institute of Microbiology, RAS, Moscow, Russia*
[4] *Department of Biotechnology Delft University of Technology, Delft, the Netherlands*

Soda lakes are defined by their high salinity and alkaline pH conditions, designating them as extreme environments. These lakes support an active microbial sulfur cycle, enhanced by the chemical stability and low toxicity of sulfide and polysulfides at these elevated pH conditions. Correspondingly, a wide variety of haloalkaliphilic anaerobes have been found in these lakes, which can use various sulfur species including elemental sulfur and thiosulfate for dissimilatory energy conservation. We investigated sulfidogenic processes of three haloalkaliphilic strains, isolated from soda lakes: *Dethiobacter alkaliphilus*, *Desulfurivibrio alkaliphilus* and *Desulfonatronospira thiodismutans*. These strains are able to conserve energy from the metabolism of sulfur compounds through reduction and disproportionation reactions. They were cultured anaerobically with different sulfuric electron acceptors and short chain organic electron donors to investigate their growth dynamics. Their genomes have been sequenced by the Joint Genome Institute and genes involved in reductive sulfur metabolisms could be identified using the IMG Data Management & Analysis system by studying the gene expression levels in the laboratory cultures. By combining physiology experiments with genomic analyses, we were able to identify metabolic abilities of these microbes and identify the sulfidogenic pathways. Comparative genomic analyses between these extremophiles are important as these experiments bring us closer to the identification of key genes responsible in sulfur transformation with special focus on disproportionation, a so far poorly understood sulfur-dependent metabolism. It will also contribute to our understanding of the degree of metabolic flexibility of haloalkaliphiles with respect to differences between sulfur disproportionation and sulfur reduction reactions. Their sulfidogenic activity in cultures also holds ecological relevance, as soda lakes contain many sulfur-oxidizers that depend on the sulfide that is produced by sulfur-reducers. Therefore, the activity and gene expression of haloalkaliphilic sulfidogens can also provide an approximation for the activity of the closed sulfur cycle in these extreme environments.

P51

The hidden sulfur cycle in rice paddy soil: identification of key sulfate reducing microorganisms by next-generation amplicon sequencing

Michael Pester[1], Jianguo Dan[2, 3], Sarah Zecchin[4, 5], Alexander Loy[4], Ralf Conrad[2]

[1] *Department of Biology, University of Konstanz, Germany*
[2] *Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[3] *College of Environment and Plant Protection, Hainan University, China*
[4] *Division of Microbial Ecology, University of Vienna, Austria*
[5] *Department of Food, Environmental and Nutritional Sciences, University of Milan, Italy*

Rice paddy fields are indispensable for human food supply but at the same time are one of the most important sources of the greenhouse gas methane. A hidden sulfur is proposed to occur in freshwater wetlands such as rice paddy fields that effectively cycles the various sulfur species between their oxidized and reduced states and at the same time counter balances methane production. Dissimilatory sulfate reduction is a major process within the hidden sulfur cycle in rice paddy soil and operates at rates comparable to marine surface sediments, despite the significantly lower sulfate concentrations. As a consequence, sulfate reduction as the thermodynamically favorable process over fermentations coupled to methanogenesis diverts organic matter degradation from methane towards more carbon dioxide production. To stimulate and thus identify the responsible microorganisms, we set up greenhouse experiments where whole rice plants were grown in soil amended with gypsum ($CaSO_4$) in amounts relevant for rice agriculture (0.15% w/w). Rice plants grown in soil without gypsum served as control. Gypsum amendment significantly reduced methane emission from rice plant mesocosms by up to 98%, showing that sulfate reducers were active and effectively competed with microorganisms involved in the methanogenic degradation pathways. 16S rRNA gene-targeted high throughput amplicon sequencing revealed a clear effect of gypsum amendment on the total microbial community in the rhizosphere and bulk soil. In particular, the abundance of members of the Desulfobulbaceae, Desulfovibrionaceae, and Synthrophobacteraceae increased under conditions that stimulated sulfate reduction. In contrast, methanotrophic bacteria belonging to the Methylococcaceae decreased in abundance, most likely as an effect of less methane supply. Our results corroborate the importance of the hidden sulfur cycle in controlling production of the greenhouse gas methane and identified key players involved in sulfate reduction as a key process in this biogeochemical phenomenon.

P52

"Thiocyanate dehydrogenase" is a novel copper enzyme of the primary thiocyanate degradation in haloalkaliphilic sulfur-oxidizing bacterium *Thioalkalivibrio paradoxus* ARh1

Stanislav I. Tsallagov[1], Tamara V. Tikhonova[1], Dimitry Y. Sorokin[2, 3], Arnulf Kletzin[4], Vladimir O. Popov[1]

[1] *A.N.Bach Institute of Biochemistry Russian Academy of Sciences*
[2] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia*
[3] *Department of Biotechnology, Delft University of Technology, Delft, The Netherlands*
[4] *Microbiology - Sulfur Biochemistry and Microbial Bioenergetics, Technische Universität Darmstadt, Darmstadt, Germany*

Thiocyanate (NCS^-) is a toxic and chemically recalcitrant compound present in natural and industrial habitats. Few lithotrophic sulfur-oxidizing bacteria are able to utilize thiocyanate as sole energy and nitrogen source. Primary thiocyanate degradation can proceed either via carbonyl sulfide (COS) or cyanate (CNO^-) as stable intermediates. The former is performed by a well characterized Co enzyme, thiocyanate hydrolase, while practically nothing is known about the enzyme(s) of the cyanate pathway of thiocyanate degradation.

Haloalkaliphilic sulfur-oxidizing bacterium *Thioalkalivibrio paradoxus* degrade thiocyanate via the cyanate pathway. The responsible enzyme, "thiocyanate dehydrogenase" (TcDH) is a copper-containing periplasmic oxido-reductase oxidizing sulfane atom of CNS^- to sulfur with formation of CNO^- as an intermediate. Ferricytochrome *c* can be used as an e-acceptor in vitro. Increasing of copper content in the growth medium stimulated the rate of thiocyanate utilization and TcDH specific activity. During purification procedure TcDH loses the copper. The process of copper dissociation is reversible. X-ray fluorescence analysis and inductively coupled plasma mass spectrometry have shown that copper content in the enzyme increases from one to four atoms per protein molecule after Cu^{2+} reconstitution. Circular dichroism showed that Cu^{2+} binding was not accompanied by considerable structural rearrangements. The process of copper incorporation was rather slow taking 2-3 days of incubation to achieve maximum enzyme activity. The increase in copper content resulted in a 100-fold increase in the enzyme activity and 4-fold decrease in the substrate affinity. Furthermore, the copper-saturated TcDH revealed increased stability during storage.

Copper-complexing compounds with the binding affinity higher than that for thiocyanate (cyanate and cyanide) inhibited TcDH. Cyanide is a competitive inhibitor. Redox-inactive metals, such as zinc, inhibited the TcDH activity, probably by replacing copper ions in the active site. Overall, the data showed a crucial role of copper in catalytic function of TcDH.

P53

Bacterial genera involved in diverse biological pathways for inorganic sulfur compounds oxidation in hypersaline soda lake brines

Charlotte Vavourakis[1], Rohit Ghai[2], Francisco Rodriguez Valera[2], Dimitry Sorokin[3, 4], Gerard Muyzer[1]

[1] Microbial Systems Ecology, Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam, The Netherlands
[2] Evolutionary Genomics Group, Departamento de Producción Vegetal y Microbiología, Universidad Miguel Hernández, San Juan de Alicante, Spain
[3] Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia
[4] Department of Biotechnology, Delft University of Technology, Delft, The Netherlands

Hypersaline soda lakes are double-extreme environments with salt concentrations up to saturation and a pH exceeding 9.5 due to the domination of sodium carbonate salts. Here we examined snapshots of the microbial community structure of four hypersaline soda lake brines in the Kulunda Steppe (Altai region, South-Eastern Siberia, Russia) with salinities varying between 170 and 400 g/L using direct (PCR-independent) metagenome sequencing. In parallel, we used amplicon sequencing targeting environmental 16S rRNA gene fragments to obtain a genus-level OTU distribution within the most abundant bacterial taxa. Analysis of the directly sequenced reads encoding 16S rRNA gene fragments revealed the dominant abundance of Euryarchaeota in the hypersaline brines with a minimum salt concentration of 250 g/L. In the brine with salinity 170 g/L bacterial phyla of Bacteroidetes, Gamma- and Alphaproteobacteria were dominant. A significant fraction of the bacterial OTUs assigned to the amplicon sequences belonged to various groups of Alpha- and Gammaproteobacteria known to be involved in the oxidative part of the microbial sulfur cycle. The most dominant genus in the brine with the lowest salinity was *Thioalkalivibrio*, a diverse group of chemolithoautrophic sulfur oxidizing bacteria (SOB). In the same brine an important fraction of the alphaproteobacterial amplicon sequences belonged to *Roseinatronobacter*, a unique genus of haloalkaliphilic, aerobic bacteriochlorophyll *a*–containing (ABC) bacteria capable of lithoheterotrophic growth by oxidation of reduced sulfur compounds to sulfate. Although Gammaproteobacteria were only low abundant in the saturated brine (salinity 400 g/L), we still could detect amplicon sequences from both purple sulfur bacteria from the genus *Halorhodospira* and SOB from the genus *Thioalkalivibrio*. Finally, *Halomonas* was the most important gammaproteobacterial genus in this brine, suggesting that there is a potential for bacterial heterotrophic thiosulfate oxidation to tetrathionate even at saturating salt conditions.

P54

Single cell genomics provides hints into the unexpected roles of the widely distributed *Dehalococcoidia* (DEH), phylum Chloroflexi, in marine subsurface sulfur cycling

Kenneth Wasmund[1, 2], Myriel Cooper[1], Lars Schreiber[3], Karen G. Lloyd[3], Dorthe G. Petersen[3], Andreas Schramm[3], Ramunas Stepanauskas[4], Richard Reinhardt[5], Bo Barker Jørgensen[3], Alexander Loy[2], Lorenz Adrian[1]

[1] *Helmholtz Centre for Environmental Research – UFZ, Germany*
[2] *Division of Microbial Ecology, University of Vienna, Austria*
[3] *Center for Geomicrobiology, Aarhus University, Denmark*
[4] *Bigelow Laboratory for Ocean Sciences, USA*
[5] *Max Planck Genome Centre Cologne, Germany*

Bacteria of the class *Dehalococcoidia* (DEH), phylum Chloroflexi, are globally distributed in shallow and deep marine sediments. Despite the prevalence of DEH in the marine subsurface, little is known about their metabolic properties or roles in biogeochemical cycles. In this research, genomic content from 5 single cells of the DEH were obtained from sediments of Aarhus Bay, Denmark, and analysed in order to predict key metabolic properties. In one single cell, we identified a gene cluster encoding for dissimilatory sulfite reductase (Dsr). This suggests some DEH have the capacity to reduce sulfite, although genes that indicate the source of sulfite, were not recovered in the corresponding genome. The single cell provides the first report for genes encoding Dsr in the phylum Chloroflexi and the first phylogenetic identity for a clade of unknown Dsr-harbouring organisms known from molecular surveys of *dsrAB*. We also provided further genetic evidence for the potential for sulfite reduction by other putative DEH taxa and at other sites by amplifying genomic fragments containing the *dsr* gene cluster directly from sediment-derived DNA. Several genes were also identified in multiple single cells that encode oxidoreductases of the complex iron-sulfur molybdoenzyme (CISM) family. These were phylogenetically affiliated with CISM enzymes known to reduce dimethyl sulfoxide, suggesting various DEH could use these molecules as electron acceptors. Genes encoding enzymes with organo-sulfur hydrolysis activity also hinted to the roles of DEH in desulfonating sulfonated organic compounds. Together, this data provides indications that DEH may play various previously unknown roles in sulfur cycling within the marine subsurface.

P55

Anaerobic oxidation of methane using different sulphur compounds as electron acceptors in a bioreactor

Chiara Cassarini[1, 2], Eldon R. Rene[1], Graciela Gonzalez-Gil[1], Piet N. L. Lens[1]

[1] UNESCO-IHE Institute for Water Education, Westvest 7, 2611 AX Delft, The Netherlands
[2] Helmholtz Centre for Environmental Research - UFZ, Permoserstraße 15, 04318 Leipzig, Germany

Anaerobic oxidation of methane coupled to sulphate reduction (AOM-SR) is a known natural process occurring in anaerobic environments, but the mechanism has not yet been fully understood. AOM investigation have another research direction; the desulphurization of industrial wastewater using methane as the sole electron donor. However, the slow growing nature of anaerobic methanotrophs (ANME) remains a major challenge for AOM-SR practical applications. This research focuses on the development of a bioprocess for AOM using alternative sulphur compounds as electron acceptors, on the characterization of the biomass, on the identification of the factors controlling the growth of the microorganisms involved and on the optimization of the design for biotechnological application.

The slow microorganisms have been enriching in bioreactors with high biomass retention capability, using marine sediments as inocula to facilitate microbial growth. Small sized biotrickilng filter (BTF) reactors (0.4 L) were used in order to select the most suitable sulphur compounds as electron acceptor for methane oxidation. Sulphate, elemental sulphur and thiosulphate were used as electron acceptors, while methane as electron donor. In each reactor, sulphide, sulphate, thiosulphate, methane and carbon dioxide were monitored. The reactor with sulphate showed complete sulphate consumption and sulphide production along with the enrichment of ANME identified by FISH (fluorescence in situ hybridization). The reactors with thiosulphate and elemental sulphur showed disproportionation to sulphide and sulphate. Sulphide production was less than expected for all the reactors suggesting formation of other sulphur compounds. Incubations with ^{13}C-labeled methane will be done to determine the AOM rate. The selected electron acceptor will be tested in the 5 L BTF reactor, by varying different process parameters. The microorganisms' phylogenetic identity will be correlated to the metabolic activity by FISH-NanoSIMS. A mathematical model for the BTF, sensitivity analysis and cost-benefit analysis will be applied to investigate the bioreactors performance and application.

P56

A sulphur reducer isolated from Tinto River, an acid rock drainage environment

Anna Patrícya Florentino[1, 2], Jan Weijma[2], Alfons J. M. Stams[1, 3], Irene Sánchez-Andrea[1]

[1] *Laboratory of Microbiology, Wageningen University, The Netherlands*
[2] *Sub-department of Environmental Technology, Wageningen University, The Netherlands*
[3] *CEB-Centre of Biological Engineering, University of Minho, Portugal*

Sulphidogenesis, especially from sulphate, is widely applied for bioremediation and metal recovery of acidic streams from mining and metallurgical activities. Application of elemental sulphur reduction instead of sulphate reduction may also be attractive as 4 times less electron donor is needed to form the same amount of sulphide. We enriched and isolated microorganisms able to perform sulphur reduction at low pH from sediments of an extremely acidic environment, Tinto River, Spain. Sediments were incubated at 30°C and pH 2 to 5 with hydrogen, glycerol, methanol, and acetate as electron donors. Sulphur-reducing activity was obtained at a minimal pH of 3 with hydrogen as electron donor and a pH of 4 with acetate. Cloning and sequencing of the 16S rRNA showed for both substrates dominance of the deltaproteobacterial sulphur-reducing genus *Desulfurella*. Similar sequences have been detected in 16S rRNA pyrosequencing of the sediment used as inoculum and in other studies of acidic environments. We combined different traditional isolation methods such as serial dilutions, antibiotic treatment and developed a novel anaerobic agar-medium with colloidal sulphur. A pure culture of *Desulfurella* sp. strain TR1 was obtained. A few features of the isolate link it back to its isolation source especially growth in a pH range from 3 to 6.5, and its metal tolerance: The sensitivity of *Desulfurella* sp. strain TR1 to heavy metals was in the inhibitory order of Pb > Zn > Cu > Ni with Ni inhibiting at 1.7 mM and Pb at 0.04 mM, suggesting the ability of the strain to cope with high metal concentration and its suitability to recover metals from acidic streams.

P58

Assessing the role of sulfide-oxidizing nitrate-reducing Epsilonproteobacteria in oil field corrosion

Sven Lahme, et al.

School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK

Microbiologically-influenced corrosion (MIC) of metal infrastructure is a multibillion £ problem. In the oil industry MIC is often linked to the production of hydrogen sulfide (H_2S) by sulfate (SO_4^{2-})-reducing microorganisms (SRM), e.g. due to injection of sulfate-rich seawater into oil reservoirs to maintain in-situ pressure. Nitrate (NO_3^-) injection, as a 'green' bioengineering strategy, is often used to counteract souring by promoting: i) organotrophic nitrate-reducing microorganism (oNRM), competing with SRM for oil organic carbon sources, ii) sulfide-oxidizing NRM (soNRM), consuming the souring agent H_2S and iii) NRM-mediated production of nitrite (NO_2^-), as a potent SRM inhibitor. However, soNRM can produce corrosive sulfur intermediates such as elemental sulfur (S^0), thiosulfate ($S_2O_3^{2-}$) or polysulfides (S_n-S^{2-}). Epsilonproteobacterial soNRM are frequently detected in oil reservoirs and have been linked to MIC during souring control by nitrate injection. The oil-field soNRM Sulfurimonas sp. strain CVO initially converts sulfide to elemental sulfur prior to further oxidation to SO_4^{2-}. NO_3^--dosing regimes at sour oil fields could therefore affect soNRM metabolism and influence corrosion. Strain CVO was incubated with iron coupons at different initial N/S ratios ranging from 6.0–0.9. High corrosion rates of 0.23–0.27 mm/y were observed when nitrate was high relative to sulfide (ratios 6.0–1.5) whereas at a lower relative nitrate dose (ratio 0.9) corrosion was only 0.14 mm/y. Time-dependent examination of corrosion coupons revealed initially corrosion at a high rate (0.37-0.48 mm/y), which decreased around 80% after 50 h (0.08-0.09 mm/y). The high initial rate coincided with maximal abundance of S^0 and therefore it may play a key role in the observed high corrosion of strain CVO. *Sulfurimonas* spp. contain *sox* genes for oxidation of reduced sulfur compound, and their expression may govern the differential accumulation of S intermediates in response to varying nitrate dose regimes.

P59

Isolation, characterization and genome analysis of a nitrate reducing *Arcobacter* sp. isolated from injection water

Irene Roalkvam[1], Karine Drønen[2], Runar Stokke[1], Håkon Dahle[1], Ida Helene Steen[1]

[1] *University of Bergen, Department of Biology, Norway*
[2] *University of Bergen, UniResearch, Norway*

Corrosion of mild steel is a pervasive problem in the oil industry, and the replacement of pipes and installations is costly. One of the best studied bacterial groups associated with microbially induced corrosion (MIC) are the sulfate-reducing bacteria (SRB), while less is known about the other microbial taxa involved in this process. To expand our knowledge on this, a nitrate reducing *Arcobacter* strain was isolated from the Oilfield A in the North Sea, where the saline aquifer water added to the injection water results in a great corrosion potential. Physiological and metabolic characterization of the *Arcobacter* isolate revealed a mesophilic microorganism capable of respiration with nitrate, elemental sulfur, ferric iron and oxygen (3-10% O_2). Organic substrates such as acetate, lactate, peptone, pyruvate, tryptone, xylan and yeast extract are utilized, as well as H_2, H_2S, elemental sulfur and thiosulfate. The genome included genes for flagellar motility, chemotaxis and biofilm formation, in addition to genes encoding metabolic pathways corresponding to the substrates mentioned above. A complete *sox* system and sulfide:quinone oxidoreductase allowes for oxidation of sulfur species, and presence of genes for polysulfide reductase and tetrathionate reductase suggests that elemental sulfur and tetrathionate could be used as terminal electron acceptors. The genome analyses also revealed an incomplete denitrification pathway (nitrate reduced to nitrite), a NAD^+-reducing hydrogenase (Hox) and an enzyme complex for dissimilatory iron reduction; thereby expanding our knowledge of the metabolic properties of Epsilonproteobacteria. Overall, the *Arcobacter* strain has a great MIC potential as a result of its capacity for biofilm formation and wide metabolic range. A direct role in MIC includes production of corrosive agents like nitrite, H_2S and sulfuric acids; while an indirect role includes maintaining favorable growth conditions for SRB by O_2 removal, detoxification of H_2S and providing small fatty acids from degradation of complex organic substrates.

P60

Combined H_2S and thiols removal from sour gas streams at haloalkaline conditions

Pawel Roman[1, 2], Martijn Bijmans[2], Albert Janssen[1, 3]

[1] Sub-department of Environmental Technology, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands
[2] Wetsus, Centre of Excellence for Sustainable Water Technology, Oostergoweg 7, 8911 MA Leeuwarden, The Netherlands
[3] Shell Technology Centre Bangalore, RMZ Centennial Campus B, Kundalahalli Main Road, Bengaluru 560 048, India

Hydrogen sulfide (H_2S) is the main sulfur pollutant in fuel gasses. The release of sulfur compounds to the atmosphere is unwanted because of air pollution and acid deposition. Besides H_2S, volatile organic sulfur compounds (VOSC's) can be present in sour gasses, which comprise toxicity, malodorous and negative environmental impact.

Nowadays, removal of H_2S and VOSC's from sour gas streams is of a great urge to reduce SO_2 emission. Treatment of sour gas streams can be achieved by a variety of well-known physicochemical processes. Main drawbacks of these processes are the high costs for operation especially for small-size treatment energy use has a large contribution. Next to the power consumption, relative high costs for chemicals, catalysts and disposal of physicochemical processes can be overcome by applying biological processes.

A two-step biological treatment process is often used to remove hydrogen sulfide from sour gas streams. In the first step, H_2S is absorbed in a mildly alkaline solution; in the second step, sulfur-oxidizing bacteria oxidize under oxygen-limiting conditions the hydrogen sulfide ions (HS^-) to elemental sulfur also referred as 'biosulfur'.

Previous research focused on developing a biological treatment processes for H_2S removal from gaseous and liquid stream. The aim of this project is to develop a process in which will be possible remove not only H_2S but also methanethiol and higher thiols from gas streams. In addition to that, we will investigate the effect of thiols on sulfur oxidizing bacteria present in desulfurization systems. Our research demonstrates that it is possible to remove more than 50 vol.% methanethiol in a system where a scrubber is integrated with a bioreactor that is operated at haloalkaline conditions whilst at the same time maintain meeting high selectivity for sulfur production.

P62

Dimethyldisulfide degradation by anaerobic microorganisms at haloalkaline conditions

João Sousa[1, 2], Agnes Jánoska[2], Martijn Bijmans[2], Alfons Stams[1], Caroline Plugge[1, 2]

[1] Laboratory of Microbiology, Wageningen University, Dreijenplein 10, 6703 HB Wageningen, The Netherlands
[2] Wetsus, European centre of excellence for sustainable water technology, Oostergoweg 9, 8911 MA Leeuwarden, The Netherlands

Besides inorganic sulfur compounds, like sulfate and thiosulfate, organic sulfur compounds, such as dimethylsulfide, dimethyldisulfide and methanethiol, are also present in nature. These compounds are biologically produced and can be biologically degraded anaerobically by sulfate reducers or methanogens (Van Leerdam et al 2008). Degradation of dimethyldisulfide and methanethiol in a biotechnological process at haloalkaline conditions was previously studied (Van Leerdam et al 2008). In that study, methanethiol conversion was mainly performed by methanogens, producing sulfide plus methane. The addition of methanol as extra e-donor resulted in increased degradation of dimethyldisulfide and methanethiol. However, in some cases H_2 might be a preferred e-donor since it can be produced on site. As methanethiol degrading methanogens studied thus far are all methylotrophs that do not use H_2/CO_2, using H_2 as extra e-donor is a challenge (Jones et al 1998). Thus far, no studies on dimethyldisulfide or methanethiol degradation were performed by microbial communities enriched in the presence of hydrogen. In this research two anaerobic fed-batch bioreactors were used to study dimethyldisulfide conversion with sulfate and with and without H_2 as additional electron donor. The bioreactors were operated at pH 9 and 1.5 M Na^+ and the inoculum was composed of a mixture of sediments originating from soda lakes, salt production ponds and sludge from sulfide oxidizing reactors operated at microaerophilic conditions. We showed degradation of dimethyldisulfide, using biomass enriched with H_2. Dimethyldisulfide was converted to methanethiol, which was then degraded by both methanogens and sulfate-reducing bacteria. The bioreactor fed with H_2 as electron donor was able to resist higher dimethyldisulfide concentrations, 2.5 mM compared to 0.5 mM without H_2. Growth was only observed in the bioreactor with H_2. This shows that use of H_2 is a good strategy to increase resistance to dimethyldisulfide and methanethiol and enhance their degradation at haloalkaline conditions.

P63

Ground tire biodesulfurization process: microbial community characterization and proprieties of compounds containing bio desulfurized material

Ivan Mangili[1], Valeria Tatangelo[1], Paola Caracino[2], Marina Lasagni[1], Giuseppina Bestetti[1], Andrea Franzetti[1]

[1] *Department of Earth and Environmental Sciences, University of Milano-Bicocca, Italy*
[2] *Pirelli Labs S.p.A., Italy*

The strains *Gordonia desulfuricans* 213E and *Rhodococcus* sp. AF21875 are bacteria with desulfurizing capability; they were tested in a ground tire (GTR) biodesulfurization process. Two different bioreactors were set up in order to carry out the process in a controlled environmental system. A community fingerprinting, automated ribosomal inter-genic spacer analysis (ARISA), was conducted on samples collected after different times during the experiment to detect the persistence of the inoculated bacteria and to compare the community of bioreactors. Furthermore, the abundance of total bacteria (16S rRNA) and biodesulfurization potential (*dszA*) were estimated through qPCR. The community fingerprinting analysis showed the persistence of *G. desulfuricans* 213E, on the other hand the persistence was not confirmed for *Rhodococcus* sp. AF21875 due to the presence of the same ARISA fragments found also in the untreated GTR. Furthermore, a change in the community was observed in two bioreactors. In particular, the communities tended to became similar to the untreated GTR community. Nevertheless, in the bioreactors an increase of *dszA* was observed. This could be an indication of bacterial natural selection having this ability to desulfurize GTR. The desulfurized GTR by each bacterium was blended into fresh natural rubber at a concentration of 10 part per hundred of rubber (phr) to find out the devulcanized rubber with the highest compatibility for compounding and revulcanization. The rheological and mechanical properties of the compounds were investigated and compared to a compound containing untreated GTR. The results showed that the biological process led to an increase of the mechanical and rheological properties of vulcanizates containing biodesulfurized GTRs.

P65

The sulfur-iron interplay and its role in the fate of carbon in salt marsh sediment

Gilad Antler, Jennifer V. Mills, Alexandra V. Turchyn

Department of Earth Sciences, University of Cambridge, Cambridge CB2 3EQ, UK

The carbon budget at Earth's surface determines Earth's climate; this is because the partitioning of carbon among various surface reservoirs determines how much carbon dioxide is in the atmosphere, where it acts as the dominant greenhouse gas. The fate of organic carbon in shallow sediments, whether it is buried, oxidized, or made into methane, is fundamentally tied to other sensitive biogeochemical cycles such as nitrogen, iron and sulfur, through their redox couplings Salt marshes are highly productive coastal wetlands that serve a critical role in carbon sequestration and nutrient trapping. As a marginal environment poised between the terrestrial and marine realms, salt marshes are extremely vulnerable to changes in environmental conditions such anthropogenic eutrophication, climate change and sea level rise. The delicate interplay between the redox cycles of sulfur, carbon and iron, which is critical for the ultimate fate of organic carbon, can be easily unbalanced through anthropogenic change. We will present pore fluid geochemical results from sediments from British salt marsh ponds. Most of the ponds studied have sediments dominated by iron-reduction, while a few ponds are fully methanic with depleted sulfate reservoirs. We will demonstrate, using sulfur and oxygen isotopes in dissolved sulfate, how this marginal marine environment demonstrates a complex interplay between the subsurface iron, sulfur and carbon cycles. We will discuss how secular variations in space and time can initiate turnover feedbacks; these feedbacks result in two distinguishable microenvironments located only few meters apart. Ultimately, we suggest that small changes in the environmental conditions can switch the ponds from one state to another, and thus that these salt marshes may become significant sources of methane if the careful balance among these geochemical species is changed.

P66

Investigating sulfur disproportionation and anaerobic oxidation of methane coupled to iron reduction

Caroline Buckner, Laura Piepgras, Anne Schwedt, Tim Ferdelman, Marcel Kuypers, Jana Milucka

Max Planck Institute for Marine Microbiology, Germany

It has been proposed that organisms capable of anaerobic oxidation of methane (AOM) are able to replace sulfate as an electron acceptor with iron and manganese oxides [1]. This metabolism appears problematic, as dissolved sulfate is easily accessible, while iron and manganese oxides are insoluble and would require some strategy for coping with these substrates. There has been no molecular evidence to date that suggests that these organisms have this ability.

However, a recent discovery has shed light on the substrates involved in AOM [2]. The archaeal partners within the consortium reduce sulfate to zero-valent sulfur, which reacts with the sulfidic environment to form disulfides that the bacteria can use as substrates for disproportionation into sulfide and sulfate.

The well-studied abiotic reaction of iron oxides with sulfide produces both elemental sulfur and disulfides [3]. In the newly discovered mechanism of AOM, the bacteria within the consortium should be able to disproportionate these substrates, creating sulfide and sulfate. The sulfate could be used to fuel AOM performed by the archaea and the sulfide could then create more substrates for the bacteria through its abiotic interaction with iron. Such a mechanism should be sustainable, a similar experiment with Sulfurospirillum deleyianum, found that the organism cycled S up to 60 times in combination with an abiotic reaction with iron oxides [4].

Therefore, the goal of this research is to determine whether the proposed interactions allow iron-dependent AOM to occur through experiments with enrichment cultures of the AOM consortia.

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P68

Sulfur metabolism in the human gut microbiota

Yuan Feng[1], Irene Sánchez-Andrea[1], Alfons J. M. Stams[1, 2], Willem M. de Vos[1, 3]

[1] Laboratory of Microbiology, Wageningen University, the Netherlands
[2] IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Portugal
[3] Departments of Veterinary Biosciences and Bacteriology and Immunology, University of Helsinki, Helsinki, Finland

Sulfur acquisition is crucial to both humans and their symbiotic gasterointestinal tract (GI tract) microbiota. The colonic sulfur-containing compounds are either inorganic (e.g. sulfate, sulfite) or organic (e.g. dietary amino acids, bile and mucins). When it is not assimilated, the end product of the anaerobic microbial degradation of sulfur-compounds is predominantly hydrogen sulfide (H_2S). Gastrointestinal H_2S is a neuromodulator and plays a critical role in controlling physiological responses such as motility and epithelial cell health. However, it has also been suggested that H_2S has a potential pathogenic role, such as in inflammatory bowel disease, which afflicts 0.1–0.5% of individuals in western countries. The exact role and fate of sulfide in the human GI tract is not clear and there are only few sulfidogenic microorganisms described that use sulfate of sulfite as terminal electron acceptor, such as *Desulfovibrio* spp. or Bilophila spp. (via taurine). However, (meta)genomic analysis indicates that there are may more microbial groups that carry genes involved in H_2S production. Hence, the lack of representative sulfidogenic species limits our understanding of this important conversion. The aim of the present research is to enrich and isolate sulfidogenic microorganisms of the anaerobic gut ecosystems, both sulfur-compound respiring and hydrolyzing ones. Fresh fecal samples were collected from a healthy donor to perform anaerobic enrichments at 37°C, pH 7.2 with different combinations of relevant electron donors (e.g. taurine, cysteine) and acceptors (sulfate or sulfite). According to the sulfide production, we selected 6 enrichment bottles for further processing. The isolation is being done by a combination of strategies such as tenfold dilution, streaking on agar plates, antibiotic treatment and pasteurization. Once the isolates are obtained, a genome-guided characterization and metabolic pathway reconstruction will be performed to link the isolated microorganisms with their role in the GI tract.

Participants

1. Anne-Catherine Ahn
University of Amsterdam, Amsterdam, Netherlands
a.c.ahn@uva.nl

2. Karthik Anantharaman
UC Berkeley, Berkeley, United States
karthik.a@berkeley.edu

3. Gilad Antler
University of Cambridge, Cambridge, United Kingdom
ga307@cam.ac.uk

4. Cherel Balkema
University of Amsterdam, Amsterdam, Netherlands
c.balkema@uva.nl

5. Tímea Balogh
University of Szeged, Szeged, Hungary
btimea@brc.hu

6. Tom Berben
University of Amsterdam, Oegstgeest, Netherlands
T.Berben@uva.nl

7. Jasmine Berg
Max Planck Institute for Marine Microbiology, Bremen, Germany
jberg@mpi-bremen.de

8. Emma Bertran
Harvard University, Cambridge, United States
emmabertran@fas.harvard.edu

9. Martijn Bijmans
WETSUS, Leeuwarden, Netherlands
Martijn.Bijmans@wetsus.nl

10. Jesper Tataru Bjerg
Aarhus University, Aarhus, Denmark
jjbjerg@bios.au.dk

11. Rich Boden
University of Plymouth, Plymouth, United Kingdom
rich.boden@plymouth.ac.uk

12. Andreas Bøggild
Aarhus University, Aarhus, Denmark
anbog@mbg.au.dk

13. Alexander Bradley
Washington University in St. Louis, St. Louis, United States
abradley@eps.wustl.edu

14. Jose Artur Brito
ITQB-UNL, Oeiras, Portugal
jbrito@itqb.unl.pt

15. Elias Broman
Linnaeus University, Kalmar, Sweden
elias.broman@lnu.se

16. Caroline Buckner
Max Planck Institute for Marine Microbiology, Bremen, Germany
cbuckner@mpi-bremen.de

17. Julea Butt
University of East Anglia, Norwich, United Kingdom
j.butt@uea.ac.uk

18. Barbara Campanini
Università di Parma, Parma, Italy
barbara.campanini@unipr.it

19. Donald Canfield
University of Southern Denmark, Odense, Denmark
dec@biology.sdu.dk

20. Diana Vasquez Cardenas
Royal Netherlands Institute for Sea Research (NIOZ), Yerseke, Netherlands
diana.vasquez@nioz.nl

21. Chiara Cassarini
UNESCO-IHE, Delft, Netherlands
c.cassarini@unesco-ihe.org

22. Christiane Dahl
University of Bonn, Bonn, Germany
ChDahl@uni-bonn.de

23. Lars Damgaard
Aarhus University, Aarhus, Denmark
lrd@bios.au.dk

24. Francesco Danza
University of Applied Sciences of Southern Switzerland (SUPSI), Bellinzona, Switzerland
francesco.danza@supsi.ch

25. Mark Dopson
Linnaeus University, Kalmar, Sweden
mark.dopson@lnu.se

26. Ágnes Duzs
University of Szeged, Szeged, Hungary
duzs.agnes@gmail.com

27. Stefan Dyksma
Max Planck Institute for Marine Microbiology, Bremen, Germany
sdyksma@mpi-bremen.de

28. Yuan Feng
Wageningen UR, Wageningen, Netherlands
yuan.feng@wur.nl

29. Timothy Ferdelman
Max Planck Institute for Marine Microbiology, Bremen, Germany
tferdelm@mpi-bremen.de

30. David Fike
Washington University in St. Louis, St. Louis, United States
dfike@levee.wustl.edu

31. Alyssa Findlay
University of Delaware, Lewes, United States
afindlay@udel.edu

32. Kai Finster
Aarhus University, Aarhus, Denmark
Kai.Finster@biology.au.dk

33. Anna Patrícya Florentino
Wageningen University, Wageningen, Netherlands
anna.florentino@wur.nl

34. Niels-Ulrik Frigaard
University of Copenhagen, Helsingør, Denmark
nuf@bio.ku.dk

35. Manabu Fukui
Hokkaido University, Sapporo, Japan
my-fukui@pop.lowtem.hokudai.ac.jp

36. Diego Giao García
Aarhus University, Aarhus, Denmark
diego.giao@biology.au.dk

37. H. Rex Gaskins
University of Illinois, Urbana, United States
hgaskins@illinois.edu

38. Antje Gittel
Aarhus University, Aarhus, Denmark
antjegittel80@gmail.com

39. Jon Graf
Max Planck Institute for Marine Microbiology, Bremen, Germany
jgraf@mpi-bremen.de

40. Marianne Guiral
CNRS-Aix-Marseille University, Marseille, France
guiral@imm.cnrs.fr

41. Bela Hausmann
University of Vienna, Vienna, Austria
bela.hausmann@univie.ac.at

42. Ian Head
Newcastle University, Newcastle upon Tyne, United Kingdom
ian.head@newcastle.ac.uk

43. Petra Henke
DSMZ, Braunschweig, Germany
phe10@dsmz.de

44. William Hocking
University of Bergen, Bergen, Norway
William.hocking@bio.uib.no

45. Rainer Höfgen
Max Planck Institute of Molecular Plant Physiology, Potsdam (OT) Golm, Germany
hoefgen@mpimp-golm.mpg.de

46. Roseanne Holanda
Bangor University, Bangor, United Kingdom
bsp40e@bangor.ac.uk

47. Casey Hubert
Newcastle University, Newcastle upon Tyne, United Kingdom
casey.hubert@newcastle.ac.uk

48. Lee Hutt
University of Plymouth, Saltash, United Kingdom
lee.hutt@students.plymouth.ac.uk

49. Marion Jaussi
Aarhus University, Aarhus, Denmark
marion.jaussi@bios.au.dk

50. Lara M. Jochum
Aarhus University, Aarhus, Denmark
jochum@bios.au.dk

51. Barrie Johnson
Bangor University, Bangor, United Kingdom
d.b.johnson@bangor.ac.uk

52. David Johnston
Harvard University, Cambridge, United States
johnston@eps.harvard.edu

53. Alexey Kamishny
Ben-Gurion University of the Negev, Beer Sheva, Israel
alexey93@gmail.com

54. Naoki Kamiya
Tokyo Metropolitan University, Hachioji, Japan
kamiya-naoki@ed.tmu.ac.jp

55. Ulrike Kappler
The University of Queensland, St. Lucia, Australia
u.kappler@uq.edu.au

56. Yoko Katayama
Tokyo University of Agriculture and Technology, Tokyo, Japan
katayama@cc.tuat.ac.jp

57. Kasper Urup Kjeldsen
Aarhus University, Aarhus, Denmark
kasperuk@bios.au.dk

58. Tobias Koch
Universität Bonn, Bonn, Germany
koch.tobias@uni-bonn.de

59. Anne-Christin Kreutzmann
Max Planck Institute for Marine Microbiology, Bremen, Germany
akreutzm@mpi-bremen.de

60. Jan Kuever
Bremen Institute for Materials Testing, Bremen, Germany
kuever@mpa-bremen.de

61. Julia Kurth
Rheinische Friedrich-Wilhelms-Universität Bonn, Bonn, Germany
j.kurth@uni-bonn.de

62. Sven Lahme
Newcastle University, Newcastle upon Tyne, United Kingdom
sven.lahme@ncl.ac.uk

63. William Leavitt
Washington University in St. Louis, St. Louis, United States
wleavitt@eps.wustl.edu

64. Silke Leimkuehler
University of Potsdam, Potsdam, Germany
sleim@uni-potsdam.de

65. Piet Lens
UNESCO-IHE, Delft, Netherlands
p.lens@unesco-ihe.org

66. Alexander Loy
University of Vienna, Vienna, Austria
loy@microbial-ecology.net

67. Samuel Luedin
University of Geneva, Basel, Switzerland
samuel.ludin@etu.unige.ch

68. Angeliki Marietou
Aarhus University, Aarhus, Denmark
angelikimarietou@gmail.com

69. Andrew Masterson
Harvard University, Belmont, United States
amasters@fas.harvard.edu

70. Emily Denise Melton
University of Amsterdam, Amsterdam, Netherlands
E.D.Melton@UvA.nl

71. Filip Meysman
Royal Netherlands Institute for Sea Research (NIOZ), Yerseke, Netherlands
filip.meysman@nioz.nl

72. Snehit Mhatre
Aarhus University, Aarhus, Denmark
snehit.mhatre@biology.au.dk

73. Hanna Miettinen
VTT Technical Research Centre of Finland Ltd, Espoo, Finland
hanna.miettinen@vtt.fi

74. Jana Milucka
Max Planck Institute for Marine Microbiology, Bremen, Germany
jmilucka@mpi-bremen.de

75. Marc Mussmann
Max Planck Institute for Marine Microbiology, Bremen, Germany
mmussman@mpi-bremen.de

76. Gerard Muyzer
University of Amsterdam, Amsterdam, Netherlands
g.muijzer@uva.nl

77. Gaofeng Ni
Linnaeus University, Kalmar, Sweden
gaofeng.ni@lnu.se

78. Lars Peter Nielsen
Aarhus University, Aarhus, Denmark
lpn@bios.au.dk

79. Signe B. Nielsen
Aarhus University, Aarhus, Denmark
signe.brokjaer@bios.au.dk

80. Joerg Overmann
Leibniz-Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany
andrea.follmer-hirt@dsmz.de

81. Lex Overmars
University of Amsterdam, Amsterdam, Netherlands
lovermars@gmail.com

82. Christina Pavloudi
Hellenic Centre for Marine Research, Heraklion, Greece
cpavloud@hcmr.gr

83. Claus Pelikan
University of Vienna, Vienna, Austria
pelikan@microbial-ecology.net

84. Andre Pellerin
McGill University, Montreal, Canada
andrepellerin@gmail.com

85. Inês Cardoso Pereira
ITQB/Universidade Nova de Lisboa, Oeiras, Portugal
ipereira@itqb.unl.pt

86. Michael Pester
University of Konstanz, Konstanz, Germany
Michael.Pester@uni-konstanz.de

87. Jillian Petersen
Max Planck Institute for Marine Microbiology, Bremen, Germany
jmpeters@mpi-bremen.de

88. Nils Risgaard-Petersen
Aarhus University, Aarhus, Denmark
nils.risgaard-Petersen@biology.au.dk

89. Irene Roalkvam
University of Bergen, Bergen, Norway
irene.roalkvam@geobio.uib.no

90. Pawel Roman
Wageningen University, Leeuwarden, Netherlands
pawel.roman@wetsus.nl

91. Hidehiro Sakurai
Kanagawa University, Hiratsuka, Japan
sakurai@waseda.jp

92. Irene Sánchez-Andrea
Wageningen University, Wageningen, Netherlands
irene.sanchezandrea@wur.nl

93. Ana Laura Santos
Bangor University, Bangor, United Kingdom
bsp41b@bangor.ac.uk

94. André Santos
ITQB-UNL, Oeiras, Portugal
andresantos@itqb.unl.pt

95. Hendrik Schaefer
University of Warwick, Coventry, United Kingdom
H.Schaefer@warwick.ac.uk

96. David Schleheck
University of Konstanz, Konstanz, Germany
david.schleheck@uni-konstanz.de

97. Barbara Schoepp-Cothenet
CNRS, Marseille, France
schoepp@imm.cnrs.fr

98. Andreas Schramm
Aarhus University, Aarhus, Denmark
andreas.schramm@bios.au.dk

99. Lars Schreiber
Aarhus University, Aarhus, Denmark
lars.schreiber@bios.au.dk

100. Heide Schulz-Vogt
Leibniz Institute for Baltic Sea Research Warnemuende (IOW), Rostock, Germany
heide.schulz-vogt@io-warnemuende.de

101. Alexander Slobodkin
Winogradsky Institute of Microbiology RAS, Moscow, Russia
aslobodkin@hotmail.com

102. Galina Slobodkina
Winogradsky Institute of Microbiology RAS, Moscow, Russia
gslobodkina@mail.ru

103. João Sousa
Wageningen University, Leeuwarden, Netherlands
joao.sousa@wetsus.nl

104. Valeria Tatangelo
University of Milano-Bicocca, Milano, Italy
v.tatangelo@campus.unimib.it

105. Bo Thamdrup
University of Southern Denmark, Odense, Denmark
bot@biology.sdu.dk

106. Vera Thiel
The Pennsylvania State University, State College, United States
vut1@psu.edu

107. Casper Thorup
Aarhus University, Aarhus, Denmark
caat@gmx.net

108. Mauro Tonolla
University of Applied Sciences of Southern Switzerland (SUPSI), Bellinzona, Switzerland
mauro.tonolla@supsi.ch

109. András Tóth
University of Szeged, Szeged, Hungary
totha@brc.hu

110. Stanislav Tsallagov
Bach Institute of Biochemistry RAS, Moscow, Russia
s.i.tsallagov@gmail.com

111. Charlotte Vavourakis
University of Amsterdam, Amsterdam, Netherlands
charlottevavourakis@gmail.com

112. Sofia Venceslau
ITQB-UNL, Oeiras, Portugal
sofiav@itqb.unl.pt

113. Michael Visser
Wageningen University, Wageningen, Netherlands
michael.visser@wur.nl

114. Carsten Vogt
Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany
carsten.vogt@ufz.de

115. Kenneth Wasmund
University of Vienna, Vienna, Austria
wasmund@microbial-ecology.net

116. Hannah Sophia Weber
University of Southern Denmark, Odense, Denmark
hannah@biology.sdu.dk

117. Boswell Wing
McGill University, Montreal, Canada
boswell.wing@mcgill.ca

118. Xiaofen Wu
Linnaeus University, Kalmar, Sweden
xiaofen.wu@lnu.se