

R2K Data Compliance Plan

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Collaborative Research: Microbiology and Biogeochemistry of Autotrophic Microbes in the Subsurface at Hydrothermal Vents: Filamentous-Sulfur Producing Bacteria

Field programs to date:

RESET06 – AT15-6 (June 16 – July 7, 2006); Karen von Damm, chief scientist; our program contributed 2 dives to rapid response cruise

AT15-15 (January 10 – February 5, 2007); Tim Shank, chief scientist; invited to participate

Fix08 – AT15-28* (December 26, 2007 – January 20, 2008); Stefan Sievert, chief scientist

Fix08_2 – AT15-38* (October 13 – November 5, 2008); Stefan Sievert, chief scientist

*Cruises dedicated towards goals of grant

Aim of program

At deep-sea vents, chemolithoautotrophic microbes mediate the transfer of energy from the geothermal source to the higher trophic levels. These organisms acquire energy from the oxidation of reduced inorganic compounds and are able to fix inorganic carbon, providing a continuous supply of organic carbon for heterotrophs and higher trophic levels. However, presently our knowledge on the identity and activity of these microorganisms at vents is inadequate (Sievert et al., 2007; Sievert et al., 2008a). To address the question of how much carbon is produced in diffuse flow areas and to identify the responsible organisms, this project pursued a multifaceted approach combining analytical chemistry, isotopic analysis, organic geochemistry, microbiological methods, and genomic approaches.

Besides the two cruises funded through this grant (AT15-28, AT15-38), we were able to obtain samples from two additional cruises. The first cruise was a rapid response cruise that was organized after the eruption at 9° N EPR (RESET06, June 2006). Our program contributed two dives to this cruise, as it was directly related to our primary objective to investigate the role of filamentous-sulfur forming microbes in the formation of so-called snow blower vents, a feature that has been previously observed in response to an eruption at this site. In addition, we had the opportunity to participate in cruise AT15-15 in January 2007 (invitation by Tim Shank and Costa Vetriani). Thus, we have samples from 6-9 months to ~34-37 months after the eruption.

Specific analyses on datasets

While significant progress has been made in assessing the extent of microbial diversity at deep-sea vents, our understanding of the functioning of these microbially-driven ecosystems is clearly less developed (Bach et al., 2006; Sievert et al., 2007; Sievert et al., 2008a). Our data will help narrowing this gap by providing a comprehensive assessment of autotrophic processes and the controlling factors at a particular vent site. Thus, our study can be seen as a case study that will provide important information and input parameters for modeling efforts to arrive at a more global assessment of chemoautotrophic carbon production. There are also efforts underway by other PIs to obtain similar information at other deep-sea vent sites. By comparing emerging datasets, we will be able to better understand differences and similarities between vent sites, which ultimately will result in a more thorough understanding of chemoautotrophic production and its controls. Our data will also contribute by better defining the extent and nature of the subsurface biosphere, which remains one of the least understood components of deep-sea hydrothermal vent ecosystems. Lastly, our data

on microbial colonization and succession (Gulmann et al., in prep.) can be integrated with information on faunal community members as well as geological and geochemical data, contributing to a more holistic view of how biological communities respond to perturbations, such as volcanic eruptions. Specifically, we have performed the analyses listed below.

1. Microbial Ecology of diffuse-flow hydrothermal vents

We have obtained total cell counts from ~10 diffuse-flow vent sites at 9°N on the EPR. The cell numbers are generally in the range of $2-5 \times 10^5$ cells/ml, and thus significantly elevated compared to the ambient deep-sea water. We are also determining the abundance of specific microbial groups of bacteria in these samples by using catalyzed reporter deposition fluorescent in situ hybridization (CARD-FISH). These analyses are ongoing, but first results confirm the dominance of epsilonproteobacteria in these vent fluids. It is important to note that there are not many data on cell numbers and even less on group specific cell counts in diffuse-flow hydrothermal fluids, an important parameter to assess the productivity and ecological importance of these systems.

We have pursued a metagenomic approach to obtain information on the metabolic potential and adaptations of the microbial communities contained in diffuse-flow vent fluids. So far most investigations have focused on assessments of microbial diversity based on 16S rRNA analyses, and less effort has been placed on obtaining functional information. The data obtained so far provide an unprecedented insight into functional aspects of the communities, allowing us to not only determine which metabolic pathways are potentially present, but also to assess their relative importance by analyzing gene frequencies. So far our efforts have been focusing on two vent sites with contrasting chemistry, and we are beginning to reveal the underlying mechanisms that drive these communities. For example, the analyses indicate that oxidation of reduced S-compounds coupled to either oxygen or nitrate reduction are the predominant pathways for energy generation at these two sites, with some site specific differences that might be attributable to differences in physicochemical conditions. These reactions are being carried out by epsilonproteobacteria, in particular members of the genera *Sulfurimonas* and *Sulfurovum*, that use the Sox- pathway for sulfur oxidation and the reductive TCA cycle for carbon fixation (Hügler et al., 2007; Sievert et al., 2008b). The metagenomic analyses also provide further evidence for the importance of sulfide-oxidizing *Arcobacter* species, whose capacity to form filamentous sulfur has been further elucidated as part of this grant (Sievert et al., 2007). The metagenomic analyses are being complemented by 16S rRNA based diversity assessments, as well as analyses of intact polar lipids and other lipid biomarkers.

To further gain insights into which microbes are actively performing autotrophic production and to obtain rates of carbon fixation, shipboard incubations utilizing ^{14}C - and ^{13}C -labelled bicarbonate under varying conditions were performed at CrabSpa and Lvent. At both sites, highest carbon fixation rates were found close to the in situ temperature. However, significant activity was still observed at 70°C, indicating the presence of organisms that might perform carbon fixation at elevated temperatures in the seafloor. The data clearly show that the main energy sources for carbon fixation at diffuse flow vents are sulfur- and hydrogen oxidation either with oxygen or nitrate. To identify chemoautotrophic microbes we are further using techniques that are based on the incorporation of ^{13}C -labeled compounds. The analyses of these incubations are currently being conducted by WHOI/MIT joint program student Kevin Richberg. The results of these studies will aid us in gaining a more comprehensive understanding of autotrophic processes at deep-sea hydrothermal vents, and the obtained data will also provide critical information to better constrain the potential importance of the sub-seafloor biosphere as a contributory factor to the overall productivity and functioning of deep-sea hydrothermal vents.

2. Microbial colonization of new surfaces

The occurrence of the eruption provided a unique opportunity to study the recolonization of the vents sites by microbes, something that was not feasible after the observed eruption at 9°N EPR in 1992. After an eruption, microbial biofilms are often the first visible evidence of biotic recolonization and are thought to be important precursors for invertebrate colonizers. However, the nature of microbial recolonization and succession is poorly understood. This work is done in collaboration with a number of investigators, including Costa Vetriani at Rutgers University, Stace Beaulieu and Tim Shank at WHOI, Kang Ding and Bill Seyfried at University of Minnesota, George Luther at the University of Delaware, and it includes sampling natural basalt as well as experimental approaches. Hydrothermal vent communities are regularly disturbed by vent eruptions, leaving fresh basalt where abundant animal and microbial communities once thrived. We have conducted one of the first studies on short-time scales (4-293 days) to look at initial microbial colonization in the context of vent chemistry. Our studies show that microorganisms rapidly colonize newly exposed surfaces at sites where warm water (~12-30°C) emanates from the sub-seafloor. Furthermore there appears to be a succession of microbes, with epsilonproteobacteria and in particular *Arcobacter* species as primary colonizers. Over the course of 9 months, epsilonproteobacteria decreased in relative abundance and gamma- and deltaproteobacteria increased as a proportion of the total diversity. No Archaea were detected in these samples. Additionally, the eruption at 9°N allowed us to compare our experimental results with natural recolonization patterns. A 16S rDNA clone library constructed with genomic DNA extracted from basalt approximately 5-8 months post-eruption was remarkably similar to that from our experimental panel exposed for 9 months. Based on this similarity, we suggest that vent microbial biofilms may undergo reproducible succession patterns. This work is currently being prepared for a manuscript for submission within the next 2 months. We are in the process of analyzing more basalt samples from subsequent cruises.

Our group has submitted metadata to the Ridge 2000 Data Management Office (DMO) for cruises AT15-28 and AT15-38. Derived data sets listed in table (see below) will be linked to these data sets once analyses are complete. The sites from which analyses have been carried out will be identified on the Marine Geoscience Data System (MGDS). Sample requests can be made to Stefan Sievert (ssievert@whoi.edu) or Roger Summons (rsummons@mit.edu).

References relevant to the PIs work above

- Sievert, S.M., M. Hügler, C. O. Wirsén, and C. D. Taylor. 2008a. Sulfur oxidation at deep-sea hydrothermal vents. Pp 238-258 In "Microbial Sulfur Metabolism", C. Dahl & C. G. Friedrich (eds), Springer, Berlin, Germany. ISBN-13 978-3-540-72679-1
- Sievert S. M., K. M. Scott, M. G. Klotz, Chain PS, Hauser LJ, Hemp J, Hügler M, Land M, Lapidus A, Larimer FW, Lucas S, Malfatti SA, Meyer F, Paulsen IT, Ren Q, Simon J, USF Genomics Class. 2008b. The genome of the epsilonproteobacterial chemolithoautotroph *Sulfurimonas denitrificans*. Applied and Environmental Microbiology, 74:1145-1156.
- Hügler, M., H. Huber, S. J. Molyneaux, C. Vetriani, and S. M. Sievert. 2007. Autotrophic CO₂ fixation via the reductive tricarboxylic acid cycle in different lineages within the phylum *Aquificae*: Evidence for two ways of citrate cleavage. *Environmental Microbiology* 9:81-92.

- Sievert, S. M., E. B. A. Wieringa, C. O. Wirsén, C. D. Taylor. 2007. Growth and mechanism of filamentous-sulfur formation by *Candidatus Arcobacter sulfidicus* in opposing oxygen-sulfide gradients. *Environmental Microbiology* 9:81-92.
- Markert S., C. Arndt, H. Felbeck, R. A. Feldman, D. Belcher, S. M. Sievert, M. Hügler, D. Albrecht, J. Robidart, S. Bench, M. Hecker, and T. Schweder. 2007. Approaching the uncultivable endosymbiont of *Riftia pachyptila* by physiological proteomics. *Science* 315:247-250.
- Sievert, S. M., R. Keene, and H. Schulz. The sulfur cycle. 2007. The Oceanography Society June 07 special issue "A Sea of Microbes" edited by Lita Procter and David Karl.
- Bach, W., K. E. Edwards, J. M. Hayes, J. A. Huber, S. M. Sievert, and M. L. Sogin. 2006. Energy in the Dark: Fuel for Life in the Deep Ocean and Beyond. *EOS Transactions, American Geophysical Union*, Vol. 87, No. 7

Anticipated products

- Gulmann, L. K., S. E. Beaulieu, T. M. Shank, K. Ding, W. E. Seyfried, S. M. Sievert. Microbial succession at 9°N on the East Pacific Rise. In prep.
- Sievert S. M., C. D. Taylor, S. C. Schuster, L. K. Gulmann. Pathways of carbon and energy metabolism of diffuse-flow deep-sea hydrothermal vent communities revealed by metagenomic analyses. In prep.

Additional results related to this program

- Voordeckers J. W., M. Do, M. Hügler, V. Ko, S. M. Sievert, and C. Vetriani. 2008. Culture dependent and independent analyses of 16S rRNA and ATP citrate lyase genes: a comparison of microbial communities from different black smoker chimneys on the Mid-Atlantic Ridge. *Extremophiles* 12:627-640.
- Scott, K. M., S. M. Sievert et al. 2006. The Genome of Deep-Sea Vent Chemolithoautotroph *Thiomicrospira crunogena* XCL-2. *PLoS Biology*. Vol. 4, No. 12, e383 doi:10.1371

Data set	Metadata	Specific Analyses	Storage	Status/Delivery Date	References/responsible PI
In situ large volume pump samples	From Alvin dives during 4 cruises: 2006 (AT15-6), 2007 (AT15-15), 2008 (AT15-28, AT15-38)	DNA and in some cases lipids were extracted from filters for microbial community analyses; metagenome of two samples has been obtained by direct 454 pyrosequencing	Frozen at -80°C; sequence data on computer and back up on external hard drive	Metagenome analyses expected to be submitted for publication by 06/2010; lipid analyses ongoing, expected to be completed by 06/2010; further DNA and protein based analyses ongoing, expected to be completed by 12/2010	Sievert, Summons

SIP incubations with ¹³ C-labeled bicarbonate	From Alvin dives during 2 cruises: 2008 (AT15-28, AT15-38)	Microbial community analyses to identify chemoautotrophic community members	Frozen at -80°C and preserved in PBS/EtOH at -20°C	Analyses are in progress and a first publication is expected to be submitted by the end of 2010.	Sievert (work by WHOI/MIT JP student Kevin Richberg)
Incubations with ¹⁴ C-labeled bicarbonate	From Alvin dives during 1 cruise: 2008 (AT15-28)	Determination of rates of autotrophic carbon fixation	Vials at RT; counts stored on computer and back up on external hard drive	Analyses are ongoing; 12/2010	Sievert, Taylor
Gene sequences	Used pure cultures obtained in other cruises/Pis	Phylogenetic analyses	GenBank	n/a	Hügler et al., 2007 Sievert
Purification of symbionts from Riftia and Tevnia	From Alvin dives during 2 cruises: 2008 (AT15-28, AT15-38)	Proteomic and genomic analyses on symbionts	Frozen at -80°C	08/2011 (analyses being carried out w/o support from grant)	Felbeck, Schweder/Markert
Basalt colonization	From Alvin dives during 4 cruises: 2006 (AT15-6), 2007 (AT15-15), 2008 (AT15-28, AT15-38)	Microbial community analyses	Frozen at -80°C and preserved in PBS/EtOH at -20°C	DNA extracted from basalt from Reset06 (AT15-6) and compared with dataset of colonization experiment prior to eruption, work to be submitted within next 3 months; other samples are being processed; 08/2011 for final integrated data	Sievert Gulmann et al., in prep
Physico-chemistry	From Alvin dives during 2 cruises: 2008 (AT15-28, AT15-38)	In situ analyses (pH, H ₂ S, temp); shipboard and shore-based analyses (volatile inorganic (sulfide, DIC, CO, H ₂ , Mg, Na, K, Ca, Li) and organic compounds (methane, acetate, methanethiols) in diffuse flow and focused flow fluids		12/2011 (analyses being carried out w/o support from grant); data will be integrated with microbiological analyses	Le Bris (in situ chemistry and major samplers) Seewald (isobaric samplers)