Total microbial cell densities and 16S rRNA abundance from North Pond from the Maria S. Merian cruise MSM20-5 in 2012 (North Pond Microbes project)

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Project
» Collaborative Research: Characterization of Microbial Transformations in Basement Fluids, from Genes to Geochemical Cycling (North Pond Microbes)

Programs
» Center for Dark Energy Biosphere Investigations (C-DEBI)
» International Ocean Discovery Program (IODP)

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Dataset Description

Total microbial cell densities and 16S rRNA abundance from IODP boreholes, seawater, and drilling mud at North Pond.

Methods & Sampling

Crustal fluids were collected from the single horizon at U1382A and from the shallow, middle, and deep horizons at U1383C using an ROV-based pumping and filtration system tailored for microbial sampling. The mobile pumping system (or MPS) is described in:
Cowen, J. P. et al. 2012. Advanced instrument system for real-time and time-series microbial geochemical sampling of the deep (basaltic) crustal biosphere. Deep-Sea Research Part I: Oceanographic Research Papers 61, 43-56, doi:10.1016/j.dsr.2011.11.004

Individual CORK fluid delivery lines were flushed using the MPS at a rate of ~4 liters per minute for at least 3 times volume of the fluid delivery line (~20-30 minutes) prior to diverting fluid flow to six 15 L foil-lined sample bags (Jensen Inert Products) that were acid cleaned and sterilized using gamma irradiation prior to deployment. Fluids were not sampled until at least 15-30 minutes of stable, reproducible measurements were observed, indicating a fully flushed fluid delivery system and access to crustal fluids. In addition, ~5 L of fluid from each of the three horizons in U1383C was filtered in situ onto 47 mm SUPOR filters in pancake-style filter

holders (McLane Inc). These filtered samples were preserved in situ using a reservoir of RNA Later (Qiagen) that is part of the MPS pumping system. Once recovered, ten liters of each sample was filtered onto a 0.22 um Sterivex-GP filter at 5 degrees C for microbial analysis. Similarly, bottom seawater was collected by CTD at 100 m above the sea floor and filtered in the same manner. In situ and ship-based filters were fixed at 4 degrees C for 18 hours with RNA Later immediately after filtering or upon recovery, then frozen at -80 degrees C until nucleic acid extractions. B. Orcutt provided a frozen sample of drilling mud from U1382A from IODP Exp. 336.

Sterivex filters and 47 mm flat filters were cut into two equal pieces using sterile technique. Total genomic DNA was extracted from one half using a phenol chloroform method as previously described in:
Sogin, M. L. et al. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". Proceedings of the National Academy of Sciences of the United States of America 103, 12115-12120, doi:10.1073/pnas.0605127103

RNA was extracted from the other half with a mirVana miRNA isolation kit (Ambion Inc) preceded by a bead beating step using RNA Powersoil beads (MoBio). Extracted RNA was treated with Turbo DNase (Ambion Turbo DNA-free kit) and converted to cDNA with an Applied Biosystems (ABI) High Capacity RNA to cDNA kit prior to amplicon library preparation. Total genomic DNA was extracted from approximately 1 g of drilling mud using a MoBio UltraClean® Soil DNA Isolation Kit.

Whole crustal fluids and bottom seawater were fixed with 3.7% formaldehyde for cell counts. Up to 19.8 ml of fixed fluids were filtered onto a 0.2 um GTBP polycarbonate filter (Millipore Inc), stained with DAPI (4',6'-diamidino-2-phenylindole; Sigma), and counted via epiflourescent microscopy. For fluorescence in situ hybridization (FISH), cells were filtered onto 0.2 um GTTP polycarbonate filters (Millipore Inc) and fixed with 2% paraformaldehyde, rinsed with milliQ H2O, air dried and stored at –20 degrees C until further use. Cells on filters were hybridized with HRP-labeled 16S rRNA targeted oligonucleotide probes EUB338 21, ARCH915 22 and NON338 21 (Biomers GmbH, Ulm, Germany), and the signal was amplified using Alexa 488® tyramides (Invitrogen). The permeabilization step of the protocol before probe hybridization was modified, such that the cells on the filters were first permeabilized with Proteinase K (0.005 U ul –1 in 0.05 M EDTA, 0.1 M Tris-HCl, at pH 8) for 30 minutes at 37 degrees C. Filters were then washed in 50 ml 1X PBS at room temperature, followed by a second permeabilization treatment with Lysozyme (106 U ml–1, in 0.05 M EDTA, 0.1 M Tris-HCl, at pH 8) for 30 minutes at 37 degrees C. After signal amplification, all cells were counterstained with DAPI and counted via epiflourescent microscopy.

The relative abundance of bacterial and archaeal 16S rRNA genes was determined by qPCR assays as previously described (Nadkarni, M. a., Martin, F. E., Jacques, N. a. & Hunter, N. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148, 257-266, doi:10.1128/JCM.40.5.1698 (2002) and Takai, K. & Horikoshi, K. Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes Rapid Detection and Quantification of Members of the Archaeal Community by Quantitative PCR Using Fluorogenic Probes. Applied and environmental microbiology 66, 5066-5072, doi:10.1128/AEM.66.11.5066-5072.2000). To generate standards, plasmid DNA was extracted from Axial Seamount low-temperature diffuse vent clone libraries, purified, and linearized using the WizardPlus SV Minipreps DNA Purification System (Promega Inc). Standards were constructed by mixing equal amounts of four bacterial plasmids for the quantification of bacterial 16S rRNA gene and two archaeal plasmids for the quantification of archaeal 16S rRNA gene. A 1:10 dilution series of the plasmid mixtures beginning with an initial concentration of 0.06 ng/ul (bacteria) and 0.10 ng/ul (archaea) was used to produce standard curves with R2 values > 0.991 and with efficiency ranging from 93 to 96%. Each 20 ul reaction contained KAPA PROBE FAST ABI Prism® 2X qPCR Master Mix (Kapa Biosystems Inc), forward and reverse primers at optimized concentrations of either 3 nM (bacteria) or 4 nM (archaea), optimized probe concentrations of either 2.5 nM (bacteria) or 5.0 nM (archaea), DEPC-treated water, and 2 ul of DNA template. Triplicate reactions were performed on a StepOne Plus Real Time PCR System (Applied Biosystems Inc) for each sample and for no template controls. Cycles began with initial denaturation for 3 min at 96 degrees C, followed by 40 cycles of 15 s at 96 degrees C and 3 min at 59 degrees C. STEPONE software version 2.2.2 (Applied Biosystems Inc) was used to analyze the results.

Data Processing Description

+/- 95% confidence levels for total cell counts are reported. Standard deviations are reported for average 16S rRNA gene copies per ng DNA.

BCO-DMO Data Processing:
- modified parameter names to conform with BCO-DMO naming conventions;

- separated confidence intervals into separate columns;
- separated lat/lon into separate columns;
- changed lon from positive degrees west to negative degrees east;
- converted values from scientific notation;
- replaced 'na' with 'nd' to indicate "no data";
- created depth min and max columns; reformatted depth range column;
- moved "in situ" text from avg_16S column to comment;
- replaced spaces with underscores in site name.

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Data Files

File	
NP_Biomass_Huber_2012.csv	(Comma Separated Values (.csv), 777 bytes) MD5:81c7d6f613fc3374f31e5660a8d66ce8
Primary data file for dataset ID 568460	

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Parameters

Parameter	Description	Units
sample	Sample name.	text
cells_per_mL	Number of cells per milliliter of fluid.	cells per milliliter (mL)
cells_per_mL_CI	95% confidence level of cells_per_mL.	cells per milliliter (mL)
rel_abund_hybridized	Relative abundance of cells hybridized with bacteria-specific probe EUB33 (%).	percentage (%)
rel_abund_hybridized_CI	95% confidence level of rel_abund_hybridized_CI	percentage (%)
avg_16S_rRNA_gene_copies	Average 16S rRNA gene copies per nanogram (ng) DNA.	Avg per nanogram (ng) DNA
avg_16S_rRNA_gene_copies_stdev	Standard deviation of avg_16S_rRNA_gene_copies.	Avg per nanogram (ng) DNA
collection_date	Date sample was collected.	mm/dd/yyyy
lat	Latitude of sample collection. Positive = North.	decimal degrees
lon	Longitude of sample collection. Negative = West.	decimal degrees
depth_mbsf_range	Depth range of sample.	meters below seafloor (mbsf)
depth_mbsf_min	Minimum depth of sample.	meters below seafloor (mbsf)
depth_mbsf_max	Maximum depth of sample.	meters below seafloor (mbsf)
comment	Text indicating if "in situ" label was noted in the "avg_16S_rRNA_gene_copies" column of original dataset.	text

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Deployments

MSM20-5

Website	https://www.bco-dmo.org/deployment/555399
Platform	R/V Maria S. Merian
Report	http://dmoserv3.whoi.edu/data_docs/Huber/Fahrtbericht_MSM20_5_02.pdf
Start Date	2012-04-11
End Date	2012-05-10

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Project Information

Collaborative Research: Characterization of Microbial Transformations in Basement Fluids, from Genes to Geochemical Cycling (North Pond Microbes)

Coverage: North Pond, mid-Atlantic Ridge

Description from NSF award abstract:
Current estimates suggest that the volume of ocean crust capable of sustaining life is comparable in magnitude to that of the oceans. To date, there is little understanding of the composition or functional capacity of microbial communities in the sub-seafloor, or their influence on the chemistry of the oceans and subsequent consequences for global biogeochemical cycles. This project focuses on understanding the relationship between microbial communities and fluid chemistry in young crustal fluids that are responsible for the transport of energy, nutrients, and organisms in the crust. Specifically, the PIs will couple microbial activity measurements, including autotrophic carbon, nitrogen and sulfur metabolisms as well as mineral oxide reduction, with quantitative assessments of functional gene expression and geochemical transformations in basement fluids. Through a comprehensive suite of in situ and shipboard analyses, this research will yield cross-disciplinary advances in our understanding of the microbial ecology and geochemistry of the sub-seafloor biosphere. The focus of the effort is at North Pond, an isolated sediment pond located on ridge flank oceanic crust 7-8 million years old on the western side of the Mid-Atlantic Ridge. North Pond is currently the target for drilling on IODP expedition 336, during which it will be instrumented with three sub-seafloor basement observatories.

The project will leverage this opportunity for targeted and distinct sampling at North Pond on two German-US research cruises to accomplish three main objectives:

1. to determine if different basement fluid horizons across North Pond host distinct microbial communities and chemical milieus and the degree to which they change over a two-year post-drilling period.

2. to quantify the extent of autotrophic metabolism via microbially-mediated transformations in carbon, nitrogen, and sulfur species in basement fluids at North Pond.

3. to determine the extent of suspended particulate mineral oxides in basement fluids at North Pond and to characterize their role as oxidants for fluid-hosted microbial communities.

Specific outcomes include quantitative assessments of microbial activity and gene expression as well as geochemical transformations. The program builds on the integrative research goals for North Pond and will provide important data for guiding the development of that and future deep biosphere research programs. Results will increase understanding of microbial life and chemistry in young oceanic crust as well as provide new insights into controls on the distribution and activity of marine microbial communities throughout the worlds oceans.

There are no data about microbial communities in ubiquitous cold, oceanic crust, the emphasis of the proposed work. This is an interdisciplinary project at the interface of microbial ecology, chemistry, and deep-sea oceanography with direct links to international and national research and educational organizations.

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Program Information

Center for Dark Energy Biosphere Investigations (C-DEBI)

Website: http://www.darkenergybiosphere.org

Coverage: Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins.

C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet?

C-DEBI's scientific goals are pursued with a combination of approaches:
(1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites;
(2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems;
(3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and
(4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists.

Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI.

Data Management:
C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their Data Management Plan (PDF) and in compliance with the NSF Ocean Sciences Sample and Data Policy. The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation.

To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

International Ocean Discovery Program (IODP)

Website: http://www.iodp.org/index.php

Coverage: Global

The International Ocean Discovery Program (IODP) is an international marine research collaboration that explores Earth's history and dynamics using ocean-going research platforms to recover data recorded in seafloor sediments and rocks and to monitor subseafloor environments. IODP depends on facilities funded by three platform providers with financial contributions from five additional partner agencies. Together, these entities represent 26 nations whose scientists are selected to staff IODP research expeditions conducted throughout the world's oceans.

IODP expeditions are developed from hypothesis-driven science proposals aligned with the program's science plan *Illuminating Earth's Past, Present, and Future*. The science plan identifies 14 challenge questions in the four areas of climate change, deep life, planetary dynamics, and geohazards.

IODP's three platform providers include:

- The U.S. National Science Foundation (NSF)
- Japan's Ministry of Education, Culture, Sports, Science and Technology (MEXT)
- The European Consortium for Ocean Research Drilling (ECORD)

More information on IODP, including the Science Plan and Policies/Procedures, can be found on their website at http://www.iodp.org/program-documents.

A summary table with links to IODP datasets currently hosted on Zenodo (https://zenodo.org/communities/iodp) can be accessed using the following link: https://iodp.tamu.edu/database/zenodo.html

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1061934
NSF Division of Ocean Sciences (NSF OCE)	OCE-1062006

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