Reconstructed genomes from North Pond, western flank of the Mid-Atlantic Ridge, from 2012-2014

Website: https://www.bco-dmo.org/dataset/782058
Data Type: Other Field Results
Version: 1
Version Date: 2019-12-02

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)

Contributors	Affiliation	Role
Huber, Julie	Marine Biological Laboratory (MBL)	Principal Investigator
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Abstract
Reconstructed genomes from North Pond

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Dataset Description

Crustal fluids were collected from the single horizon at U1382A and from the shallow, middle and deep horizons in U1383C (Edwards et al., 2012) using a mobile pumping system designed for microbial sampling from CORK fluid delivery lines as described in Meyer et al. (2016) and Cowen et al. (2012). Deployed with the ROV system, mobile pumping system connectors are attached to the CORK wellhead via an umbilical to the hydrological zone of interest within the aquifer. In 2012, 12 l of each fluid sample were filtered on to a 0.22 µm Sterivex-GP filter (Merck Millipore, Billerica, MA, USA) as described in Meyer et al. (2016). In 2014, 12 l of each sample was filtered in situ and immediately fixed with RNALater (Thermo Fisher Scientific, Waltham, MA, USA), as described previously (Akerman et al., 2013). After sampling in 2012, a battery-powered GeoMICROBE sled was left at each CORK for time series autonomous sampling of the fluid delivery lines (Cowen et al., 2012). For each filter sample, ~10 l of fluid were filtered in situ and immediately fixed with RNALater. Sleds were deployed in April 2012 and recovered in April 2014 with samples collected. Upon sled recovery, filters were transferred to fresh RNALater and stored at −80 °C, while all bag samples were stored at 4 °C (Cowen et al., 2012). Deep bottom water was sampled in 2012 and 2014 via a CTD at 100 m above the seafloor and filtered in the same manner as the crustal fluids onto Sterivex filters. Total microbial biomass in fluids was enumerated with DAPI (4′,6′-diamidino-2-phenylindole; Sigma-Aldrich, St Louis, MO, USA) and epifluorescent microscopy (Porter and Feig, 1980).

Methods & Sampling

Ribosomal rRNA identification and relative abundance
From the high-quality paired-end Illumina sequencing reads, 16S rRNA gene fragments were identified using Meta-RNA (Huang et al., 2009; v.H3; -e 1e-10). Putative rRNA fragments and associated mate pairs from each sample were processed through EMIRGE (Miller et al., 2011, 2013); emirge_amplicon.py; -l 113 -i 163 -s 33 -a 32 —phred33) to generate full-length sequences using the SILVA (Quast et al., 2012) SSURef111 reference database (https://github.com/csmiller/EMIRGE). Reconstructed 16S rRNA genes were assigned taxonomy using mothur (v1.34.4) by first aligning the sequences to the SILVA SSURef123 database (align.seqs; flip=T), removing sequences that failed to align, if necessary (remove.seqs), and classifying the sequences (classify.seqs; cutoff=80, iters=1000).

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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Data Files

File
recon_genomes.csv (Comma Separated Values (.csv), 17.60 KB) MD5:6c06b73e963be413fe38953c8ae3d54d Primary data file for dataset ID 782058

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Related Publications

Akerman, N. H., Butterfield, D. A., & Huber, J. A. (2013). Phylogenetic diversity and functional gene patterns of sulfur-oxidizing subseafloor Epsilonproteobacteria in diffuse hydrothermal vent fluids. Frontiers in Microbiology, 4. doi:10.3389/fmicb.2013.00185
Methods

Cowen, J. P., Copson, D. A., Jolly, J., Hsieh, C.-C., Lin, H.-T., Glazer, B. T., & Wheat, C. G. (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
Methods

Edwards, K. J., Wheat, C. G., Orcutt, B. N., Hulme, S., Becker, K., Jannasch, H., … Klaus, A. (2012). Design and deployment of borehole observatories and experiments during IODP Expedition 336, Mid-Atlantic Ridge flank at North Pond. Proceedings of the IODP. doi:10.2204/iodp.proc.336.109.2012
Methods

Huang, Y., Gilna, P., & Li, W. (2009). Identification of ribosomal RNA genes in metagenomic fragments. Bioinformatics, 25(10), 1338–1340. doi:10.1093/bioinformatics/btp161
Methods

Meyer, J. L., Jaekel, U., Tully, B. J., Glazer, B. T., Wheat, C. G., Lin, H.-T., … Huber, J. A. (2016). A distinct and active bacterial community in cold oxygenated fluids circulating beneath the western flank of the Mid-Atlantic ridge. Scientific Reports, 6(1). doi:10.1038/srep22541
Methods

Miller, C. S., Baker, B. J., Thomas, B. C., Singer, S. W., & Banfield, J. F. (2011). EMIRGE: reconstruction of full-length ribosomal genes from microbial community short read sequencing data. Genome Biology, 12(5). doi:10.1186/gb-2011-12-5-r44
Methods

Miller, C. S., Handley, K. M., Wrighton, K. C., Frischkorn, K. R., Thomas, B. C., & Banfield, J. F. (2013). Short-Read Assembly of Full-Length 16S Amplicons Reveals Bacterial Diversity in Subsurface Sediments. PLoS ONE, 8(2), e56018. doi:10.1371/journal.pone.0056018
Methods

Porter, K. G., & Feig, Y. S. (1980). The use of DAPI for identifying and counting aquatic microflora. Limnology and Oceanography, 25(5), 943–948. doi:10.4319/lo.1980.25.5.0943
Methods

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. doi:10.1093/nar/gks1219
Methods

Tully, B. J., Wheat, C. G., Glazer, B. T., & Huber, J. A. (2017). A dynamic microbial community with high functional redundancy inhabits the cold, oxic subseafloor aquifer. The ISME Journal, 12(1), 1–16. doi:10.1038/ismej.2017.187
General

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Parameters

Parameter	Description	Units
bioproject_id	NIH bioproject term	unitless
Assembly	GenBank assembly accession	unitless
Level	contig or scaffold	unitless
WGS	Whole Genome Shotgun Submission	unitless
BioSample	BioSample accession numbers	unitless
Isolate	Genome designation (eg. NORP is NORthPond)	unitless
Taxonomy	Sepcies assigned with mothur for reconstructed 16SrRNA genes	unitless

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Instruments

Dataset-specific Instrument Name	Aanderaa sensor
Generic Instrument Name	Aanderaa Oxygen Optodes
Dataset-specific Description	Fluid systems were flushed and allowed to equilibrate before sampling, and dissolved oxygen concentrations were measured during pumping using an Aanderaa sensor (Meyer et al., 2016).
Generic Instrument Description	Aanderaa Oxygen Optodes are instrument for monitoring oxygen in the environment. For instrument information see the Aanderaa Oxygen Optodes Product Brochure.

Dataset-specific Instrument Name	CTD
Generic Instrument Name	CTD - profiler
Dataset-specific Description	Deep bottom water was sampled in 2012 and 2014 via a CTD at 100 m above the seafloor and filtered in the same manner as the crustal fluids onto Sterivex filters.
Generic Instrument Description	The Conductivity, Temperature, Depth (CTD) unit is an integrated instrument package designed to measure the conductivity, temperature, and pressure (depth) of the water column. The instrument is lowered via cable through the water column. It permits scientists to observe the physical properties in real-time via a conducting cable, which is typically connected to a CTD to a deck unit and computer on a ship. The CTD is often configured with additional optional sensors including fluorometers, transmissometers and/or radiometers. It is often combined with a Rosette of water sampling bottles (e.g. Niskin, GO-FLO) for collecting discrete water samples during the cast. This term applies to profiling CTDs. For fixed CTDs, see https://www.bco-dmo.org/instrument/869934.

Dataset-specific Instrument Name	epifluorescent microscopy
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Total microbial biomass in fluids was enumerated with DAPI (4′,6′-diamidino-2-phenylindole; Sigma-Aldrich, St Louis, MO, USA) and epifluorescent microscopy (Porter and Feig, 1980).
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	GeoMICROBE sled
Generic Instrument Name	GeoMICROBE
Dataset-specific Description	After sampling in 2012, a battery-powered GeoMICROBE sled was left at each CORK for time series autonomous sampling of the fluid delivery lines (Cowen et al., 2012).
Generic Instrument Description	Integrated Ocean Drilling Program borehole CORK (Circulation Obviation Retrofit Kit) observatories provide long-term access to hydrothermal fluids circulating within the basaltic crust (basement), providing invaluable opportunities to study the deep biosphere. We describe the design and application parameters of the GeoMICROBE instrumented sled, an autonomous sensor and fluid sampling system. The GeoMICROBE system couples with CORK fluid delivery lines to draw large volumes of fluids from crustal aquifers to the seafloor. These fluids pass a series of in-line sensors and an in situ filtration and collection system. GeoMICROBE's major components include a primary valve manifold system, a positive displacement primary pump, sensors (e.g., fluid flow rate, temperature, dissolved O2, electrochemistry-voltammetry analyzer), a 48-port in situ filtration and fluid collection system, computerized controller, seven 24 V-40 A batteries and wet-mateable (ODI) communications with submersibles. This constantly evolving system has been successfully connected to IODP Hole 1301A on the eastern flank of the Juan de Fuca Ridge. Reference: Cowen, J.P., Copson, D., Jolly, J., Hsieh, C.-C., Matsumoto, R., Glazer, B.T. et al. (2012) Advanced instrument system for real-time and time-series microbial geochemical sampling of the deep (basaltic) crustal biosphere., Deep-Sea Research I, 61: 43-56 doi:10.1016/j.dsr.2011.11.004

Dataset-specific Instrument Name	automated colorimetric analysis
Generic Instrument Name	Titrator
Dataset-specific Description	Fluids also were analyzed for dissolved silicon and nitrate using automated colorimetric analysis and pH was measured with an electrode before a potentiometric titration for the determination of alkalinity (Wheat et al., 2017).
Generic Instrument Description	Titrators are instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

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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

MSM37

Website	https://www.bco-dmo.org/deployment/555401
Platform	R/V Maria S. Merian
Report	https://datadocs.bco-dmo.org/d3/data_docs/North_Pond_Microbes/msm37_cruise_rpt_downld2018-02-12.pdf
Start Date	2014-03-22
End Date	2014-04-21
Description	Conducted operations on subseafloor observatories (CORKs) installed during IODP Leg 336 to examine hydrological-geochemical-microbiological interactions in North Pond. The remotely operated vehicle (ROV) Jason II of the Woods Hole Oceanographic Institution (Woods Hole, USA) was the main operational tool.

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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-1061827
NSF Division of Ocean Sciences (NSF OCE)	OCE-1062006

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