Results from shipboard high-pressure incubations of diffuse flow vent fluids collected from the Crab Spa and Alvinella sites at East Pacific Rise during the AT26-10 expedition, Jan. 2014 (Microbial Communities at Deep-Sea Vents project)

Website: https://www.bco-dmo.org/dataset/628993
Data Type: experimental
Version: 2
Version Date: 2017-02-07

Project
» An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents (Microbial Communities at Deep-Sea Vents)

Programs
» Dimensions of Biodiversity (Dimensions of Biodiversity)
» Center for Dark Energy Biosphere Investigations (C-DEBI)

Contributors	Affiliation	Role
Foustoukos, Dionysis	Carnegie Institution for Science (CIS)	Principal Investigator
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Table of Contents

Coverage

Spatial Extent: **Lat**:9.83992 **Lon**:-104.2915
Temporal Extent: 2014-01-03 - 2014-01-22

Dataset Description

This dataset includes results from shipboard high-pressure incubations of diffuse flow vent fluids collected from the Crab Spa (9.8398º N, 104.2913º W) and Alvinella (9.8398º N, 104.2915º W) sites at East Pacific Rise during the AT26-10 oceanographic expedition in January 2014. Reported parameters include dates and time elapsed, flow rate, temperature, pressure, and pH, and concentrations of NO3, NH4, H2, H2S, CH4.

Vent fluids used in shipboard incubations were corrected from diffuse flow vent sites at the East Pacific Rise (2503 m): Crab Spa (9.8398º N, 104.2913º W) and Alvinella (9.8398º N, 104.2915º W) (see description in McNichol et al. [2016]). Fluids were collected using isobaric gas-tight samplers [Seewald et al., 2002] prior to their transfer to the shipboard continuous culture system [Foustoukos and Perez-Rodriguez, 2015]. Here, high-pressure incubations (250 bars) were conducted at mesophilic (30 ºC) and thermophilic (50 ºC) conditions to constrain the function and metabolic rates of denitrifying and DNRA microbial communities residing at Crab Spa and Alvinella, respectively. To enhance the activity of nitrate-respiring anaerobic bacteria, an NO3- (5 mm) and H2(aq) (1.30 mM)-enriched medium was introduced in the high-pressure incubations

under strictly anaerobic conditions. Dissolved HCO3- (7.3 mm, 13C labeled) was used as added carbon source. Vent fluids were introduced at a flow rate of 0.042 mL/min, while growth medium was added at a rate of 0.0042 mL/min. The two sets of experiments were performed for 356 (Crab Spa) and 50 hours (Alvinella). Direct cell counts were conducted by staining cells with 0.1% acridine orange and counting them with a fluorescence microscope. 15N/14N isotopic analysis of the NO3-, NH4+ and biomass were conducted with a Thermo Scientific Delta VPlus mass spectrometer and CE Instruments NA 2500 series elemental analyzer (EA).

References:

Foustoukos, D., and I. Perez-Rodriguez (2015), A continuous culture system for assessing microbial activities in the piezosphere, Applied and Environmental Microbiology, 81(19), 6850-6856.

McNichol, J., S. P. Sylva, F. Thomas, C. D. Taylor, S. M. Sievert, and J. S. Seewald (2016), Assessing microbial processes in deep-sea hydrothermal systems by incubation at in situ temperature and pressure, Deep Sea Research Part I: Oceanographic Research Papers, 115, 221-232.

Seewald, J. S., K. W. Doherty, T. R. Hammar, and S. P. Liberatore (2002), A new gas-tight isobaric sampler for hydrothermal fluids, Deep-Sea Research, Part I: Oceanographic Research Papers, 49(1), 189-196.

Methods & Sampling

From AT26-10 cruise report (01/29/2014):
DOB: An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents
Cruise Report by the CIW research team: Dr. Ileana Perez-Rodriguez, Mr. Matt Rawls and Dr. Dionysis I. Foustoukos
The CIW team was responsible for the shipboard continuous culturing incubations of vent fluids collected from Crab Spa and Tica hot springs during the AT26-10 expedition at 9oN EPR by utilizing our high-pressure bioreactor (Fig. 1). This was accomplished through a collaborative effort with Jeff Seewald and Sean Sylva (WHOI), who deployed isobaric gas-tight samplers (IGTs) to collect hydrothermal vent fluids at the diffuse flow sites. Experiments were designed to study the cycling to N through the metabolic processes of denitrification and dissimilatory nitrate reduction to ammonia (DNRA) under in-situ deep-sea vent temperature and pressure conditions.

We studied the evolution of nitrate reducing microorganisms at mesophilic (30oC) and thermophilic (50oC) conditions at pressures ranging from 5 to 250 bar. Vent fluids (16 IGTs) were delivered in the bioreactor and homogeneously mixed with aqueous media solution enriched in dissolved nitrate, hydrogen and 13C labeled bicarbonate to facilitate the growth of nitrate reducing microorganisms (Fig. 2). The two distinct sets of experiments were lasted for 356 and 100 hours. In short, experimental results constrained the function and metabolic rates of the denitrifying microbial communities in the Crab Spa fluids, while DNRA metabolic pathways were identified for the populations residing in the moderate temperature vent fluids (60oC) of the Alvinella colony at Tica.

During the course of the experiments we monitored the growth of deep-sea microbial communities by measuring the concentrations of dissolved aqueous species directly involved in nitrate based metabolism, such as NO3, NH4, H2 and H2S. We also monitored cell densities by utilizing an epi-fluorescence microscope (Sievert, WHOI). Dissolved gas and NH4+ concentrations were attained by gas and ion chromatography (Seewald - Sylva, WHOI). Subsamples were also collected for a number of offshore analysis to determine: i) the 15N/14N isotope composition of NO3-,/NH4+ and constrain kinetic isotope effects associated with denitrification/DNRA (Perez-Rodriguez, CIW), ii) to study the rates of autotrophic carbon fixation by NanoSIMS (Musat, UFZ), iii) to perform single cell genomics on the microbial populations grown in the bioreactor (Ramunas, Bigelow) and (iv) to isolate and characterize novel microogranisms from the communities cultured in our experiments (Perez-Rodriguez, CIW and Vetriani, Rutgers).

Data Processing Description

BCO-DMO Processing:

- added conventional header with dataset name, PI name, version date
- renamed parameters to BCO-DMO standard

- concatenated the 2 datasets: Crab Spa and Alvinella patch
- blank cells replaced with 'nd'
- added columns for description, date_start and date_end
- version 2017-02-07 replaced version 2015-12-17: added cell concentration, d15N_NO3_ppt, and d15N_Biomass_ppt

[table of contents | back to top]

Data Files

File
crab_spa_incubation_v2.csv (Comma Separated Values (.csv), 4.85 KB) MD5:5cd087e56498f1bf2da15e791d26da44 Primary data file for dataset ID 628993

[table of contents | back to top]

Parameters

Parameter	Description	Units
description	description of experimental incubation	unitless
date_start	start date of incubation in yyyy-mm-dd format	unitless
date_end	end date of incubation in yyyy-mm-dd format	unitless
flow_rate	flow rate	milliliters/minute
temp	temperature	degrees Celsius
press	pressure	MegaPascals
time_elapsed	time since start of incubation	hours
NO3_uM	nitrate concentration	umoles/kgr
NH4_uM	ammonium concentration	umoles/kgr
H2_uM	hydrogen concentration	umoles/kgr
H2S_uM	hydrogen sulfide concentration	umoles/kgr
CH4_uM	methane concentration	umoles/kgr
pH	pH at 25 C	unitless
cell_concentration	cell_concentration	unknown
d15N_NO3_ppt	d15N_NO3_ppt	unknown
d15N_Biomass_ppt	d15N_Biomass_ppt	unknown

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Electron Microscope
Dataset-specific Description	JSM-6500F field emission scanning electron microscope (JEOL)
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves.

Dataset-specific Instrument Name	IGT Sampler
Generic Instrument Name	Isobaric Gas-Tight Sampler
Generic Instrument Description	Isobaric Gas Tight (IGT) samplers, designed and built by scientists and engineers at WHOI, are titanium instruments designed to be used with deep submergence vehicles to sample corrosive hydrothermal vent fluids at high temperature and high pressure. The IGT prevents the sampled fluid from degassing as pressure decreases during the vehicle's ascent to the surface.

Dataset-specific Instrument Name	
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Olympus BX61 microscope with a UPlanF1 100x (numerical aperture, 1.3) oil immersion objective
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	custom high pressure bioreactor
Generic Instrument Name	Shipboard Incubator
Dataset-specific Description	The integrated system allows for the culturing of microorganisms under hydrostatic pressures from 0.1 to 69 MPa (and up to 138 MPa with ongoing developments) and at temperatures ranging from 25 to 120°C. For full description, see Foustoukos and Perez-Rodriguez (2015), Applied and Environmental Microbiology, 81, 6850
Generic Instrument Description	A device mounted on a ship that holds water samples under conditions of controlled temperature or controlled temperature and illumination.

[table of contents | back to top]

Deployments

AT26-10

Website	https://www.bco-dmo.org/deployment/529031
Platform	R/V Atlantis
Report	http://dmoserv3.bco-dmo.org/data_docs/Microbe_Vent_Communities/AT26-10_Cruise_Report_v2_2015-07-09.pdf
Start Date	2013-12-29
End Date	2014-01-27
Description	Samples were collected by ROV Jason II at the 9N deep-sea hydrothermal vent field on the East Pacific Rise, Pacific Ocean

[table of contents | back to top]

Project Information

An Integrated Study of Energy Metabolism, Carbon Fixation, and Colonization Mechanisms in Chemosynthetic Microbial Communities at Deep-Sea Vents (Microbial Communities at Deep-Sea Vents)

Deep-sea hydrothermal vents, first discovered in 1977, are poster child ecosystems where microbial chemosynthesis rather than photosynthesis is the primary source of organic carbon. Significant gaps remain in our understanding of the underlying microbiology and biogeochemistry of these fascinating ecosystems. Missing are the identification of specific microorganisms mediating critical reactions in various geothermal systems, metabolic pathways used by the microbes, rates of the catalyzed reactions, amounts of organic carbon being produced, and the larger role of these ecosystems in global biogeochemical cycles. To fill these gaps, the investigators will conduct a 3-year interdisciplinary, international hypothesis-driven research program to understand microbial processes and their quantitative importance at deep-sea vents. Specifically, the investigators will address the following objectives: 1. Determine key relationships between the taxonomic, genetic and functional diversity, as well as the mechanisms of energy and carbon transfer, in deep-sea hydrothermal vent microbial communities. 2. Identify the predominant metabolic pathways and thus the main energy sources driving chemoautotrophic production in high and low temperature diffuse flow vents. 3. Determine energy conservation efficiency and rates of aerobic and anaerobic chemosynthetic primary productivity in high and low temperature diffuse flow vents. 4. Determine gene expression patterns in diffuse-flow vent microbial communities during attachment to substrates and the development of biofilms.

Integration: To address these objectives and to characterize the complexity of microbially-catalyzed processes at deep-sea vents at a qualitatively new level, we will pursue an integrated approach that couples an assessment of taxonomic diversity using cultivation-dependent and -independent approaches with methodologies that address genetic diversity, including a) metagenomics (genetic potential and diversity of community), b) single cell genomics (genetic potential and diversity of uncultured single cells), c) meta-transcriptomics and -proteomics (identification and function of active community members, realized potential of the community). To assess function and response to the environment, these approaches will be combined with 1) measurement of in situ rates of chemoautotrophic production, 2) geochemical characterization of microbial habitats, and 3) shipboard incubations under simulated in situ conditions (hypothesis testing under controlled physicochemical conditions). Network approaches and mathematical simulation will be used to reconstruct the metabolic network of the natural communities. A 3-day long project meeting towards the end of the second year will take place in Woods Hole. This Data Integration and Synthesis meeting will allow for progress reports and presentations from each PI, postdoc, and/or student, with the aim of synthesizing data generated to facilitate the preparation of manuscripts.

Intellectual Merit. Combining the community expression profile with diversity and metagenomic analyses as well as process and habitat characterization will be unique to hydrothermal vent microbiology. The approach will provide new insights into the functioning of deep-sea vent microbial communities and the constraints regulating the interactions between the microbes and their abiotic and biotic environment, ultimately enabling us to put these systems into a quantitative framework and thus a larger global context.

Broader Impacts. This is an interdisciplinary and collaborative effort between 4 US and 4 foreign institutions, creating unique opportunities for networking and fostering international collaborations. This will also benefit the

involved students (2 graduate, several undergraduate) and 2 postdoctoral associates. This project will directly contribute to many educational and public outreach activities of the involved PIs, including the WHOI Dive & Discover program; single cell genomics workshops and Cafe Scientifique (Bigelow); REU (WHOI, Bigelow, CIW); COSEE and RIOS (Rutgers), and others. The proposed research fits with the focus of a number of multidisciplinary and international initiatives, in which PIs are active members (SCOR working group on Hydrothermal energy and the ocean carbon cycle, http://www.scorint. org/Working_Groups/wg135.htm; Deep Carbon Observatory at CIW, https://dco.gl.ciw.edu/; Global Biogeochemical Flux (GBF) component of the Ocean Observatories Initiative (OOI), http://www.whoi.edu/GBF-OOI/page.do?pid=41475)

[table of contents | back to top]

Program Information

Dimensions of Biodiversity (Dimensions of Biodiversity)

Website: http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503446

Coverage: global

(adapted from the NSF Synopsis of Program)
Dimensions of Biodiversity is a program solicitation from the NSF Directorate for Biological Sciences. FY 2010 was year one of the program. [MORE from NSF]

The NSF Dimensions of Biodiversity program seeks to characterize biodiversity on Earth by using integrative, innovative approaches to fill rapidly the most substantial gaps in our understanding. The program will take a broad view of biodiversity, and in its initial phase will focus on the integration of genetic, taxonomic, and functional dimensions of biodiversity. Project investigators are encouraged to integrate these three dimensions to understand the interactions and feedbacks among them. While this focus complements several core NSF programs, it differs by requiring that multiple dimensions of biodiversity be addressed simultaneously, to understand the roles of biodiversity in critical ecological and evolutionary processes.

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1136608

[table of contents | back to top]