Supplementary Table 3B: Replicate cell counts for the 11 samples and alkaline phosphatase activity measurements available for any of the 11 samples

Website: https://www.bco-dmo.org/dataset/811483
Data Type: Other Field Results
Version: 1
Version Date: 2020-06-22

Project
» Collaborative Research: Delineating The Microbial Diversity and Cross-domain Interactions in The Uncharted Subseafloor Lower Crust Using Meta-omics and Culturing Approaches (Subseafloor Lower Crust Microbiology)

Program
» International Ocean Discovery Program (IODP)

Contributors	Affiliation	Role
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Abstract
Supplementary Table 3B: Overview of archaeal and bacterial lipid biomarkers and cell counts. Replicate cell counts for the 11 samples and alkaline phosphatase activity measurements available for any of the 11 samples. Samples were taken on board of the JOIDES Resolution between November 30, 2015 and January 30, 2016 in the SW Indian Ridge.

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Coverage

Spatial Extent: **Lat**:-32.70567 **Lon**:57.278183
Temporal Extent: 2015-11-30 - 2016-01-30

Dataset Description

Supplementary Table 3B: Overview of archaeal and bacterial lipid biomarkers and cell counts. Replicate cell counts for the 11 samples and alkaline phosphatase activity measurements available for any of the 11 samples. Samples were taken on board of the JOIDES Resolution between November 30, 2015 and January 30, 2016 in the SW Indian Ridge.

Methods & Sampling

Alkaline phosphatase (AP) activity was measured using the fluorogenic substrate 4-methylumbelliferyl phosphate (MUF-P) (Sigma-Aldrich, St. Louis, MO) and its reference standard, methylumbelliferone (MUF). Fluorescence was measured using black, flat bottom, 96-well microplates in a Spark 10M Multimode Microplate Reader (Tecan, Männedorf, Switzerland). Fluorescence of MUF is greatest at pH 10, therefore 25 µL of 0.4 M NaOH was added to the wells (final concentration 40 mM) to be read. 25 µL of 1M EDTA was added as well (100 mM final concentration) to prevent precipitation of c 439 arbonate from sampled veins.

Fluorescence was measured with an excitation wavelength of 380 nm and emission of 454 nm for all substrates and standards. One cm3 powdered rock was mixed with 5 cm3 of sterileartificial seawater (ASW) in a 8 mL serum vial with 90:5:5 N2:CO2:H2 headspace. 700 µL of each slurry was withdrawn with a sterile syringe to a 1.5 mL Eppendorf tube after setup but before sealing the vial; this sample served as T0, with triplicate 200 µL technical replicates. These 700 µL samples were briefly centrifuged (60 sec. at 2500 rpm) and the supernatant used for the T0 analyses.

Two additional samples were taken using the same methods as for T0 after at least 2 weeks and then again after 4-6 weeks to generate a slope of activity. Incubations were kept at 10˚C, the inferred in situ temperature, for the duration of each assay. Autoclaved, powderized rock from each of the samples was tested to determine the amount of fluorophore adsorbance to rock powder.

Adsorbance was found to behave in a systematic manner, resulting in a straight line when comparing fluorescence standards in artificial seawater (ASW) alone with fluorescence standards plus rock powder in ASW, although this relationship was found to be different when measured at 4 hours versus days later. Therefore, a correction factor for adsorbance was applied to the enzyme data for the initial measurement (t0, y=1.90x-676), taken <2 hours after experiment initiation, versus the second and third measurements (t1 and t2, y = 4.64x - 303), taken days to weeks later. Negative controls consisting of the same ASW used for the sample incubations plus substrate, but no sample, were consistently below detection. The limit of quantification for the AP assay, defined as 3X the standard deviation of the blank, was 0.0242 pmol cm-3 rock hour-1 based on analysis of eight blanks.

Data Processing Description

BCO-DMO processing notes:

- Added latitude and longitude of sample location
- Adjusted column headers to comply with database requirements

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Data Files

File
cell_counts.csv (Comma Separated Values (.csv), 725 bytes) MD5:686af120c52f0688d64fc0740971e9ee Primary data file for dataset ID 811483

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Related Publications

Coolen, M. J. L., & Overmann, J. (2000). Functional Exoenzymes as Indicators of Metabolically Active Bacteria in 124,000-Year-Old Sapropel Layers of the Eastern Mediterranean Sea. Applied and Environmental Microbiology, 66(6), 2589–2598. doi:10.1128/aem.66.6.2589-2598.2000
Methods

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Parameters

Parameter	Description	Units
Sample	Sample ID	unitless
Latitude	Latitude, south is negative	decimal degrees
Longitude	Longitude, west is negative	decimal degrees
Depth	Depth below seafloor	meters (m)
Replicate_1	Replicate 1	cells per cubic centimeter (cells/cm3)
Replicate_2	Replicate 2	cells per cubic centimeter (cells/cm3)
Average	Average number of cells	cells per cubic centimeter (cells/cm3)
Standard_Deviation	Standard deviation of average	cells per cubic centimeter (cells/cm3)
AP_activity	Alkaline Phosphatase activity	picomole per gram per hour (pmol g-1 h-1)
Time_of_AP_measurment	Time of Alkaline Phosphatase measurements	hours (Hr)

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Instruments

Dataset-specific Instrument Name	Zeiss Axio Imager M2 Epifluorescence microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Cell counts performed with a Zeiss Axio Imager M2 Epifluorescence microscope.
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	Spark 10M Multimode Microplate Reader
Generic Instrument Name	plate reader
Dataset-specific Description	Alkaline Phosphate activity was measured with a Spark 10M Multimode Microplate Reader (Tecan, Männedorf, Switzerland).
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Diagenode Bioruptor
Generic Instrument Name	ultrasonic cell disrupter (sonicator)
Dataset-specific Description	Cell separation was performed through sonication with Diagenode Bioruptor sonication device.
Generic Instrument Description	Instrument that applies sound energy to agitate particles in a sample.

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Deployments

IODP-360

Website	https://www.bco-dmo.org/deployment/810905
Platform	R/V JOIDES Resolution
Report	http://publications.iodp.org/scientific_prospectus/360/index.html
Start Date	2015-11-30
End Date	2016-01-30

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Project Information

Collaborative Research: Delineating The Microbial Diversity and Cross-domain Interactions in The Uncharted Subseafloor Lower Crust Using Meta-omics and Culturing Approaches (Subseafloor Lower Crust Microbiology)

Coverage: SW Indian Ridge, Indian Ocean

NSF abstract:

The lower ocean crust has remained largely unexplored and represents one of the last frontiers for biological exploration on Earth. Preliminary data indicate an active subsurface biosphere in samples of the lower oceanic crust collected from Atlantis Bank in the SW Indian Ocean as deep as 790 m below the seafloor. Even if life exists in only a fraction of the habitable volume where temperatures permit and fluid flow can deliver carbon and energy sources, an active lower oceanic crust biosphere would have implications for deep carbon budgets and yield insights into microbiota that may have existed on early Earth. This is all of great interest to other research disciplines, educators, and students alike. A K-12 education program will capitalize on groundwork laid by outreach collaborator, A. Martinez, a 7th grade teacher in Eagle Pass, TX, who sailed as outreach expert on Drilling Expedition 360. Martinez works at a Title 1 school with ~98% Hispanic and ~2% Native American students and a high number of English Language Learners and migrants. Annual school visits occur during which the project investigators present hands on-activities introducing students to microbiology, and talks on marine microbiology, the project, and how to pursue science related careers. In addition, monthly Skype meetings with students and PIs update them on project progress. Students travel to the University of Texas Marine Science Institute annually, where they get a campus tour and a 3-hour cruise on the R/V Katy, during which they learn about and help with different oceanographic sampling approaches. The project partially supports two graduate students, a Woods Hole undergraduate summer student, the participation of multiple Texas A+M undergraduate students, and 3 principal investigators at two institutions, including one early career researcher who has not previously received NSF support of his own.

Given the dearth of knowledge of the lower oceanic crust, this project is poised to transform our understanding of life in this vast environment. The project assesses metabolic functions within all three domains of life in this crustal biosphere, with a focus on nutrient cycling and evaluation of connections to other deep marine microbial habitats. The lower ocean crust represents a potentially vast biosphere whose microbial constituents and the biogeochemical cycles they mediate are likely linked to deep ocean processes through faulting and subsurface fluid flow. Atlantis Bank represents a tectonic window that exposes lower oceanic crust directly at the seafloor. This enables seafloor drilling and research on an environment that can transform our understanding of connections between the deep subseafloor biosphere and the rest of the ocean. Preliminary analysis of recovered rocks from Expedition 360 suggests the interaction of seawater with the lower oceanic crust creates varied geochemical conditions capable of supporting diverse microbial life by providing nutrients and chemical energy. This project is the first interdisciplinary investigation of the microbiology of all 3 domains of life in basement samples that combines diversity and "meta-omics" analyses, analysis of nutrient addition experiments, high-throughput culturing and physiological analyses of isolates, including evaluation of their ability to utilize specific carbon sources, Raman spectroscopy, and lipid biomarker analyses. Comparative genomics are used to compare genes and pathways relevant to carbon cycling in these samples to data from published studies of other deep-sea environments. The collected samples present a rare and time-sensitive opportunity to gain detailed insights into microbial life, available carbon and energy sources for this life, and of dispersal of microbiota and connections in biogeochemical processes between the lower oceanic crust and the overlying aphotic water column.

About the study area:

The International Ocean Discovery Program (IODP) Expedition 360 explored the lower crust at Atlantis Bank, a 12 Ma oceanic core complex on the ultraslow-spreading SW Indian Ridge. This oceanic core complex represents a tectonic window that exposes lower oceanic crust and mantle directly at the seafloor, and the expedition provided an unprecedented opportunity to access this habitat in the Indian Ocean.

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Program Information

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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1658031

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