Accession numbers for clone and Illumina amplicon sequence libraries from Dorado Outcrop basalts collected on R/V Atlantic cruise AT26-09 in December 2013

Website: https://www.bco-dmo.org/dataset/661056
Data Type: Cruise Results
Version: 06 October 2016
Version Date: 2016-10-06

Project
» The Dorado Outcrop low-temperature ridge flank environment (Dorado ridge flank environment)

Program
» Center for Dark Energy Biosphere Investigations (C-DEBI)

Contributors	Affiliation	Role
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Dataset Description

Accession numbers for clone and Illumina amplicon sequence libraries from Dorado Outcrop basalts collected on cruise AT26-09, ROV Jason dives J2-752 and J2-757.

Methods & Sampling

Acquisition methods are described in the following publication:
Lee, M.D., Walworth, N.G., Sylvan, J.B., Edwards, K.J., and Orcutt, B.N. 2015. Microbial Communities on Seafloor Basalts at Dorado Outcrop Reflect Level of Alteration and Highlight Global Lithic Clades. Front. Microbiol. doi:10.3389/fmicb.2015.01470

In summary (excerpted from above):
Over the span of December 7–23, 2013, 12 seafloor rock samples (11 basalts and 1 lithified carbonate, hereafter R1–R12) were collected from across Dorado Outcrop while aboard R/V Atlantis during cruise AT26-09 following previously developed protocols. Using the ROV Jason II, samples were collected and those for DNA analysis were placed in either sterile whirl-pak bags or centrifuge tubes and frozen at −80 degrees C. In addition to the basalts, two bottom water samples were collected using a Niskin bottle mounted to an elevator, and 1.25 L were filtered onto 0.2 um pore size polycarbonate Nucleopore filters that were then frozen at −80 degrees C.

DNA Extraction and Sequencing of the 16S rRNA Gene
Frozen rock pieces were crushed in a flame-sterilized impact mortar into sand-sized grains which were then transferred to sterile plastic centrifuge tubes and stored at −80 degrees C until DNA extractions were

performed. DNA extractions were carried out with the FastDNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) following the manufacturer's specifications. About 0.5 g of crushed material were placed directly into the lysis tubes of the kit, as were the bottom water filters. Protocol blanks were performed with each extraction (no samples or DNA added to lysis tubes) to track the potential for contamination. DNA concentrations were quantified with the Qubit HS dsDNA Assay kit with a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA) according to manufacturer protocols.

DNA extracts from the 12 rock samples (plus one technical replicate from the same sample), one green-colored, potential biofilm sample from R11, two bottom water samples, and four protocol blanks for a total of 20 samples were sent for DNA sequencing by a commercial vendor (Molecular Research LP; MR DNA; Shallowater, TX, USA). Illumina MiSeq paired-end (2 × 300 base pair) tag sequencing was carried out using the Earth Microbiome Project universal primers 515f and 806r, which flank the V4 region of the 16S rRNA gene. Library preparation and sequencing was carried out at the facility. In brief, the 515f/806r PCR primers with 8-base barcodes on the forward primer were used in a PCR reaction with the HotStarTaq Plus Master Mix Kit (QIAGEN:USA, Valencia, CA, USA). Based on their DNA concentrations and molecular weight, multiple samples were pooled together in equal proportions, purified with Ampure XP beads, and then used to prepare the library by following the Illumina TruSeq DNA library preparation protocol.

Sequence Data Analysis
Tag data curation and processing were carried out using mothur v.1.34.4 (Schloss et al., 2009) following the mothur Illumina MiSeq Standard Operating Procedure (Kozich et al., 2013). Refer to Lee et al (2015) for more information including treatment of extraction blanks and OTU filtering.

Clone Library Processing
For the clone library, near full-length contigs were assembled using Geneious v6.1.8 (Kearse et al., 2012). Sequences were oriented and trimmed manually and then screened for chimeras using the online program Decipher version 1.14.4 (Wright et al., 2012). The resulting sequences were used in phylogenetic tree construction and submitted to BLAST (Altschul et al., 1990) to search for nearest cultured neighbors as well as environmental samples.

Accession Numbers
The clone sequences recovered from this project are publicly available through NCBI′s GenBank, accession numbers KT748562–KT748628, and the raw tag data are available through NCBI′s Sequence Read Archive under project accession number SRP063681. Additionally, a fasta-formatted file containing the representative OTU sequences identified is available as a Datasheet 2 in Supplementary Material to Lee et al. (2015).

Data Processing Description

BCO-DMO Processing:
- BCO-DMO created file of accession numbers and links using information provided in the dataset metadata form.

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Data Files

File
dorado_basalt_seq.csv (Comma Separated Values (.csv), 12.74 KB) MD5:f5083206c162de807896a9497732acfb Primary data file for dataset ID 661056

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Parameters

Parameter	Description	Units
data_type	Type of sequence	unitless
repository	Name of repository	unitless
description	Description of the sequences	unitless
accession_number	Accession number	unitless
accession_link	Hyperlink to accession in the repository	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

AT26-09

Website	https://www.bco-dmo.org/deployment/627919
Platform	R/V Atlantis
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/AT26-09_DoradoCruiseReport2013.pdf
Start Date	2013-12-07
End Date	2013-12-23
Description	Research was conducted on this cruise as part of the C-DEBI project titled "Discovery, sampling, and quantification of flows from cool yet massive ridge-flank hydrothermal springs on Dorado Outcrop, eastern Pacific Ocean" (see: http://www.bco-dmo.org/project/627844).

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Project Information

The Dorado Outcrop low-temperature ridge flank environment (Dorado ridge flank environment)

Coverage: Seafloor basalts from Dorado Outcrop 9N 87 W

Project description obtained from C-DEBI:
With support from this project, I participated in two research cruises to the Dorado Outcrop in 2013 and 2014, leading efforts to collect samples of fluids, rocks, and sediment for microbiological characterization. In 2013, we were successful in discovering low-temperature seeps on the outcrop, although sampling issues prevented the collection of pristine fluid samples for detailed microbiological characterization. High-quality fluids were collected during the 2014 cruise, and several C-DEBI student and postdoc collaborators that participated in the 2014 cruise are currently analyzing them. We collected seafloor basalts from around the outcrop, both near and far to active venting. A student is currently analyzing 16S rRNA gene sequence libraries from these samples; preliminary analysis indicates some microbial community overlap with seafloor basalts from other deep-sea environments, but also some unique groups that may reflect the age of the Dorado Outcrop environment compared to other studies. We collected push cores and gravity cores during both cruises to document the diffusion of potentially altered basement fluids into sediment, and were successful in documenting the presence of oxygen in these cores, confirming our hypotheses about fluid-rock reactions in basement surrounding Dorado Outcrop. Several C-DEBI supported students and postdocs are further characterizing the sediment biogeochemistry and microbial community activity, structure and function. Both cruises were used as opportunities to engage the general public in deep-sea science through news articles, microblogging, and a feature on Al Jazeera America.

Related Publications:
Lee, M.D., Walworth, N.G., Sylvan, J.B., Edwards,K.J., Orcutt, B.N. 2015. Microbial Communities on Seafloor Basalts at Dorado Outcrop Reflect Level of Alteration and Highlight Global Lithic Clades. Frontiers in Microbiology 6:1470. C-DEBI Contribution 286.

Orcutt, B. N. et al. 2013. Oxygen consumption rates in subseafloor basaltic crust derived from a reaction transport model. Nat. Commun. 4:2539. C-DEBI Contribution 166.

Note: This project was funded by a C-DEBI Research Grant.

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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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0939564

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