Iberian Margin Anaerobic Sediment 18S rRNA amplicons from R/V JOIDES Resolution cruise JRES-339 in the West Iberian margin from 2011-2012 (Subseafloor Microbial Ecology project)

Website: https://www.bco-dmo.org/dataset/628004
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Project
» Genomic analyses and microbial cultivations in unexplored sub-seafloor ridge flank and continental margin environments (Subseafloor Microbial Ecology)

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

Contributors	Affiliation	Role
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Dataset Description

18S rRNA amplicons were derived from sediments from 10.5, 29.5, 47.5, 68.5, 86.5, and 123 meters below seafloor (mbsf) at IODP Site U1385A on the Iberian Margin. Amplicons were derived from extracted DNA at 10.5 and 29.5 mbsf and extracted RNA at 10.5, 29.5, 47.5, 68.5, 86.5 and 123 mbsf.

Methods & Sampling

Samples were acquired by the scientific drilling vessel JOIDES Resolution at IODP Site U1385A (37°34.285'N, 10°7.562'W) on November 25th, 2011 by Advanced Piston Coring (APC). Whole-round sections meant for molecular analysis were immediately frozen at -80 degrees C for the remainder of the cruise and shipped at this temperature. DNA was extracted using the MO BIO PowerMax Soil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) with a minor adjustment to manufacturer's protocol. Briefly, 10 g of sediment extruded from the interior (>1cm from core liner) of whole-round core samples was processed according to manufacturer's instructions, save for an additional step of incubation in a 65 degree C water bath for 15 minutes prior to step 4 (10 minute vortex). RNA was extracted using a modified protocol of the FastRNA Pro Soil-Direct Kit (MP Biomedicals, Solon, OH, USA) as described in Orsi et al. 2013b. 20 grams of thawed sediment was extracted from the center of each core whole-round sample and 5 grams placed in each of four

15 ml conical tubes. 5 mls of RNA soil lysis solution was added to each tube and vortexed for ~30 seconds. Additional soil lysis solution was added until ~ 1 ml of headspace remained. Tubes were then placed on a homogenizer (MP Biomedicals, Solon, OH, USA) and homogenized at a speed setting of 4.5 for 60 seconds. Two 15 ml tubes were combined into 50 ml conical tubes and centrifuged at 4000 rpm for 20 minutes. Supernatants were combined into one 50 ml tube. Roughly one- tenth volume of 2M sodium acetate pH 4.0 and 1 volume of phenol:chloroform pH 6.5 was added and samples were vortexed for 15 seconds and incubated at room temperature for 5 minutes. Samples were then centrifuged for 20 minutes at 4000 rpm. The top phase was removed to a new 50 ml tube. A roughly one-tenth volume of the kit's inhibitor removal solution was added and samples were again centrifuged for 5 minutes at 4000 rpm. Supernatant was poured into a new tube and 2.5 volumes of 100% molecular grade ethanol and one-tenth volume of 3M sodium acetate was added. Tubes were then briefly vortexed and allowed to precipitate overnight at -80 degrees C.

The next day, samples were spun down for 60 minutes at 4000 rpm at 4 degrees C. An additional treatment with phenol:chloroform and inhibitor removal solution was performed before a 1 hour precipitation with 100% isopropanol at -20 degrees C. Samples were centrifuged at 14,000 rpm for 20 minutes and pellet was washed with 70% ethanol and centrifuged again. Pellet was allowed to air dry and was resuspended in 200 µl of RNAse free water. A one-tenth volume of Turbo DNAse buffer (Life Technologies, Carlsbad, CA, USA) and 1 ul of Turbo DNAse was added and incubated at 37 degrees C to get rid of contaminating DNA. Tubes were centrifuged at 14,000 rpm for 2 minutes and supernatant was aspirated into a new RNAse free 1.5 ml tube. The FastRNA Pro Soil-Direct kit instructions were then followed to the end, starting at step 18 (RNA matrix/slurry step) and including the optional "centrifugation through quick- clean filters" step. PCR using DNA polymerase with 2 ul of RNA as template was performed to check for left over contaminating DNA. No DNA-based PCR product was seen (data not shown). DNA extracts (including an extraction blank) were amplified using primers for the V4 hypervariable region of the eukaryotic 18S rRNA gene (Orsi et al. 2013a). PCR was performed for 35 cycles using SpeedSTAR HS DNA polymerase (Clontech Laboratories Inc., Mountain View, CA, USA) at an annealing temperature of 63 degrees C. Reactions were performed in three separate 25 ul aliquots and pooled. Reactions were checked by gel electrophoresis and product was gel-purified using the Wizard SV Gel and PCR Cleanup system (Promega Corporation, Madison, WI, USA). Concentrations were checked via Qubit v2.0 (Life Technologies, Carlsbad, CA, USA) and equimolar aliquots of product were sent to Molecular Research LP (Shallowater, TX, USA) for barcoding and sequencing on Illumina MiSeq platform per manufacturer's instructions.

For RNA extracts (including an extraction blank), amplicons were generated with the Superscript One-Step RT-PCR system with Platinum Taq DNA polymerase (Life Technologies, Grand Island, NY, USA) using pre-barcoded primers for the V4 hypervariable region of the eukaryotic 18S rRNA gene (Orsi et al. 2013a, same core primers used for DNA amplicons). The cDNA synthesis was performed at 50 degrees C for 30 minutes, followed by 35 cycles of PCR amplification according to the manufacturer's thermocycling protocol. Triplicate 50 ul RT-PCR reactions were pooled and checked for product via gel electrophoresis. RT-PCR products were gel purified using the QIAquick Gel Purification kit (Qiagen, Netherlands) and quantified via Qubit v2.0. Equimolar quantities of barcoded RT-PCR amplicons were pooled and sent for 454 pyrosequencing with FLX Titanium chemistry according to manufacturer's instructions at Selah Genomics (Greenville, South Carolina, USA).

Data Processing Description

The raw data from DNA-derived Illumina reads (reads.full and reads.qual files) were converted to a fastq file in Qiime version 1.8 (Caporaso et. al 2010) with the convert_fastaqual_fastq.py script. Primers and barcodes were extracted with the extract_barcodes.py script and sequences were de-multiplexed via split_libraries_fastq.py and an in-house perl script. Sequences were then uploaded to the SILVA NGS pipeline (https://www.arb-silva.de/ngs, Quast et al. 2013) where default quality-trimming parameters were used, sequences were aligned with the SINA aligner (Pruesse et al. 2012), and clustering was performed at the 98% sequence identity level. Taxonomy was called against the SILVA 16S/18S rRNA database. The resulting OTU table was used for creation of taxonomy figures and further statistical analyses. The raw data from RNA-derived 454 pyrosequencing reads were denoised (denoise_wrapper.py), filtered for chimeras (ChimeraSlayer), and demultiplexed (split_libraries.py) within the Qiime environment. The resulting sequence fasta file was uploaded to the SILVA NGS pipeline and sequences were quality-trimmed, aligned, clustered, and assigned taxonomy as described above. The resulting OTU table was used for taxonomy figures and further statistical analyses. Both DNA-derived Illumina reads and RNA-derived pyrosequencing reads were deposited in the NCBI Archive under bioproject PRJNA301144. Raw reads are publicly available through the NCBI SRA via accession numbers SRR2910739-SRR2910745.

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Data Files

File
18S_rRNA.csv (Comma Separated Values (.csv), 2.14 KB) MD5:f7ec7af00f7b05bbc21574334413941b Primary data file for dataset ID 628004

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Parameters

Parameter	Description	Units
BioProject_ID	NCBI BioProject ID number.	dimensionless
organism	Type of organism.	dimensionless
BioProject_URL	Hyperlink to NCBI BioProject.	dimensionless
SRA_ID	NCBI SRA ID number.	dimensionless
sample_name	Sample name/description from NCBI.	dimensionless
description	Brief description.	dimensionless
SRA_URL	Hyperlink to NCBI SRA.	dimensionless

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Instruments

Dataset-specific Instrument Name	Advanced Piston Coring (APC)
Generic Instrument Name	Advanced Piston Corer
Dataset-specific Description	Samples were acquired by the scientific drilling vessel JOIDES Resolution at IODP Site U1385A by Advanced Piston Coring (APC).
Generic Instrument Description	The JOIDES Resolution's Advanced Piston Corer (APC) is used in soft ooze and sediments. The APC is a hydraulically actuated piston corer designed to recover relatively undisturbed samples from very soft to firm sediments. More information is available from IODP (PDF).

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

JRES-339

Website	https://www.bco-dmo.org/deployment/628013
Platform	R/V JOIDES Resolution
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/JR339_PrelimReport.pdf
Start Date	2011-11-16
End Date	2012-01-16
Description	More information is available from IODP: http://publications.iodp.org/preliminary_report/339/index.html

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Project Information

Genomic analyses and microbial cultivations in unexplored sub-seafloor ridge flank and continental margin environments (Subseafloor Microbial Ecology)

Coverage: North Pond & the Iberian Margin

Project description from C-DEBI:
Over the course of two years of C-DEBI support, I have investigated subseafloor microbial ecology in three separate environments; the basaltic crust aquifer underneath the sediments of North Pond, the sediments of North Pond, and the sediments of the Iberian Margin at IODP site U1385.

At North Pond, my research was primarily cultivation-based, with enrichments for multiple metabolisms across basalt and sediment samples. Shallow and deep heterotrophic isolates from the sediment column at site U1382B offer an opportunity to ask unique research questions regarding the breakdown of fresher, more labile organic carbon vs. older, more refractory organic carbon.

At the Iberian margin, my research was primarily molecular-based, with several enrichment and cultivation efforts initiated after compelling evidence for particular metabolisms associated with individual groups of microbes. Diversity studies using high-throughput sequencing of 16S/18S rRNA amplicons examined the distribution and abundance of bacteria, archaea, and microbial eukaryotes. To further investigate ecological

trends and the biology of particular community members, metagenomes were generated from the same DNA pools as the amplicon data.

This project was funded by a C-DEBI Graduate Student Fellowship.

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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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0939564
NSF Ocean Sciences Ocean Drilling Program (NSF OCE-ODP)	OCE-1333104

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