16S rRNA gene (from RNA) from samples collected on cruise Chikyu-331 in the Okinawa Trough, Japan from September to October 2010

Website: https://www.bco-dmo.org/dataset/781114
Data Type: Cruise Results
Version: 1
Version Date: 2019-11-05

Project
» An In-Depth analysis of the subvent biosphere within Okinawa Backarc Basin (IODP 331, Iheya North Hydrothermal Field) sediments (Subvent_Biosphere_Sediments)

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

Contributors	Affiliation	Role
House, Christopher	Pennsylvania State University (PSU)	Principal Investigator
Brandt, Leah	Pennsylvania State University (PSU)	Scientist
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Abstract
RNA extractions were attempted from sediments at Sites C0014 and C0017 to selectively investigate the active microbial populations at these sites. This study was conducted to supplement the gene-based and metagenomic studies. However, the results from sequenced reverse transcribe 16S rRNA demonstrate the sensitivity of extraction and amplification to external nucleic acids and attest to the challenges in working with low biomass environmental samples. Inconsistencies between primersets and replicates make conclusions about the results of this RNA study extremely tenuous.

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Coverage

Spatial Extent: **N**:27.791667 **E**:126.911944 **S**:27.790278 **W**:126.900556
Temporal Extent: 2010-09-16 - 2010-09-27

Dataset Description

Next-generation sequencing data across a hydrothermal gradient; Okinawa Backarc Basin IODP 331 – subvent biosphere. RNA extractions were attempted from sediments at Sites C0014 and C0017 to selectively investigate the active microbial populations at these sites. This study was conducted to supplement the gene-

based and metagenomic studies. However, the results from sequenced reverse transcribe 16S rRNA demonstrate the sensitivity of extraction and amplification to external nucleic acids and attest to the challenges in working with low biomass environmental samples. Inconsistencies between primersets and replicates make conclusions about the results of this RNA study extremely tenuous.

Methods & Sampling

Sampling of cores was done in accordance with IODP protocols.

All C0014 samples were cored on 16 September 2010 via via HPCS (hydraulic piston coring system).
All C0015 samples were cored on 18 September 2010 via HPCS.
All C0017 samples were cored on 27 September 2010 via HPCS.

Core sections were kept frozen (-80°C) until DNA extractions. RNA was extracted using a phenol chloroform protocol. The RNA extract was DNAse treated using the Invitrogen™ Ambion™ Turbo DNA-free Kit and purified using the Qiagen® RNeasy Mini Kit. A cDNA library was made using the Qiagen ® QuantiTec Reverse Transcription Kit. Polymerase chain reactions used the KAPABiosystems® KAPA2G Robust HotStart ReadyMix PCR Kit for 32 cycles.

Sequencing of the product was performed on the Illumina® MiSeq 2500.

Data Processing Description

Data Processing: Demultiplexing was performed in Mothur (v.1.30.1).

Problem report: Upon analysis, the RNA dataset does not appear to reflect the same or similar information as the published 16S rRNA gene amplicon dataset. Rather, it indicates significant background noise from extraction and not an indigenous representation of the subsurface biosphere.

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Data Files

File	
16S_rRNA_gene_from_RNA.csv	(Comma Separated Values (.csv), 2.72 KB) MD5:980f9e00f9128be44d314d806cc5cc9e
Primary data file for dataset ID 781114	

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Supplemental Files

File	
fastq files from the "Subvent Biosphere Sediments" project filename: LB_RNA_CDEBI.zip	(ZIP Archive (ZIP), 311.73 MB) MD5:d5e3046e495102534db110467f1c2477
.zip folder of fastq files from the "Subvent Biosphere Sediments" project.	
Primers used in dataset 781114 filename: Primers.csv	(Comma Separated Values (.csv), 113 bytes) MD5:417ccb8e8e5125672111cc38dfa5e5fe
Primers used in dataset 781114	

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Related Publications

Brandt, L. D., & House, C. H. (2016). Marine Subsurface Microbial Community Shifts Across a Hydrothermal Gradient in Okinawa Trough Sediments. Archaea, 2016, 1–12. doi:10.1155/2016/2690329
Results

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Parameters

Parameter	Description	Units
Cruise_ID	Cruise ID number	unitless
Sample_Name	Sample name	unitless
Latitude	Latitude; positive values = North	decimal degrees
Longitude	Longitude; positive values = East	decimal degrees
Water_depth	Water depth	meters
Top_Depth	Top depth	meters below seafloor
Bottom_Depth	Bottom depth	meters below seafloor
Sediment_type	Sediment type	unitless
Estimated_Temp	Estimated temperature; based on 3 degrees C/m	degrees Celsius
Nucleic_Acid_amplified	Type of nucleic acid amplified	unitless
Primer_Set_16S_rRNA	16S rRNA primer set	unitless
Illumina_dataset	Illumina dataset ID	unitless
Notes	Notes	unitless

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Instruments

Dataset-specific Instrument Name	hydraulic piston coring system (HPCS)
Generic Instrument Name	Advanced Piston Corer
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	Illumina MiSeq 2500
Generic Instrument Name	Automated DNA Sequencer
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

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Deployments

Chikyu-331

Website	https://www.bco-dmo.org/deployment/682963
Platform	Chikyu
Start Date	2010-09-01
End Date	2010-10-03
Description	IODP cruise for Deep Hot Biosphere expedition. For more information, including cruise reports, visit http://publications.iodp.org/proceedings/331/331title.htm

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Project Information

An In-Depth analysis of the subvent biosphere within Okinawa Backarc Basin (IODP 331, Iheya North Hydrothermal Field) sediments (Subvent_Biosphere_Sediments)

Coverage: Iheya North Hydrothermal Field, Pacific Ocean

The Iheya North Hydrothermal Field in the Okinawa Backarc Basin represents an ideal environment in which to investigate the biotic temperature fringe of microbial life at depth because of its subsurface hydrothermal activity within its continental margin-type sediment profile. Geographically, the Okinawa Backarc Basin is situated along a continental margin, which is a sediment profile type commonly sampled and studied across the seafloor (e.g. Peru Margin, Costa Rica Margin, Cascadia Margin). The hydrothermal network within the subsurface here supplies an additional temperature obstacle to microbial life existing in the sediments. In particular, the sediment profile at Site C0014 exhibits a transition from hemipelagic ooze with pumiceous volcaniclastic sediments and low temperature (4°C) to a hydrothermally altered sequence of clays within the top ~10 mbsf of sediment. Temperature measurements indicate a gradient of approximately 3°C/m, which is roughly an order of magnitude greater than continental margin sites (e.g. Cascadia Margin, IODP 311 and Costa Rica Margin, IODP 344), but is more gradual than intense, centimeter-scale gradients from other hot, surface sediments. We have focused on the application of culture-independent, molecular methods to

understand taxonomic and functional characteristics through this hydrothermal gradient. Confidence in DNA recovery suggests a microbial biosphere extent of approximately 15 mbsf (55°C).

Results from both 16S rRNA gene surveys and metagenomics analyses suggest a temperature-dependent stratigraphy of taxonomic and functional adaptations between the shallowest and deepest sample horizons. Cosmopolitan marine subsurface bacterial and archaeal taxa are present throughout the top 10 mbsf, whereas, hyperthermophilic heterotrophic as well as thermophilic anaerobic methanotrophic archaea appear in varying local abundances in deeper, hydrothermal clay horizons. "An In-Depth analysis of the subvent biosphere within Okinawa Backarc Basin (IODP 331, Iheya North Hydrothermal Field) sediments" encompasses datasets funded through C-DEBI to investigate the microbial communities in IODP 331 Iheya North Hydrothermal Field sediments. Site C0014 at this field site is 500 m away from the active vent and experiences a 3C/m temperature gradient with depth. In the research grant "Investigating the active microbial populations in near hydrothermal vent sediments" and the Research Exchange grant "Learning new RNA extraction techniques", we used an RNA-based approach to explore the active microbial community. Upon analysis, the RNA dataset does not appear to reflect the same information as the phylogenetic signals coming from both the 16S rRNA gene as well as the metagenomes. The data indicate significant background noise from the RNA extraction process rather than from an indigenous representation of the subsurface biosphere.

The graduate fellowship "An in-depth analysis of the subvent biosphere within the Okinawa backarc basin Iheya North hydrothermal field" provided an opportunity to bring together metagenomic, 16S rRNA gene amplicon (DNA), and 16S rRNA amplicon datasets from IODP 331 Iheya North Hydrothermal Field sediments to understand the biogeography of this subvent biosphere.

For more information, refer to the following C-DEBI grants/projects.
An in-depth analysis of the subvent biosphere within the Okinawa backarc basin Iheya North hydrothermal field:
https://www.darkenergybiosphere.org/award/an-in-depth-analysis-of-the-subvent-biosphere-within-the-okinawa-backarc-basin-iheya-north-hydrothermal-field/

Investigating the active microbial populations in near hydrothermal vent sediments: https://www.darkenergybiosphere.org/award/investigating-the-active-microbial-populations-in-near-hydrothermal-vent-sediments/

Learn new RNA extraction techniques: https://www.darkenergybiosphere.org/award/learn-new-rna-extraction-techniques/

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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-0939564

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