16S rRNA gene amplicon sequence accessions collected from the R/V JOIDES Resolution (JRES-204) cruise in the Cascadia Continental Margin during 2002 (Methane-Hydrate Sediment Omics project)

Website: https://www.bco-dmo.org/dataset/626821
Version: 2015-12-02

Project
» Functional gene diversity and expression in methane-hydrate bearing deep subsurface sediments (Methane-Hydrate Sediment Omics)

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

Contributors	Affiliation	Role
Glass, Jennifer B.	Georgia Institute of Technology (GA Tech)	Principal Investigator
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Dataset Description

This dataset was generated under a C-DEBI research grant.

Related References:

Tréhu, A.M, Bohrmann, G., Rack, F.R., Torres, M.E., et al., 2003. Proc. ODP, Init. Repts., 204: College Station, TX (Ocean Drilling Program).doi:10.2973/odp.proc.ir.204.2003

Shipboard Scientific Party, 2003. Site 1244. In Tréhu, A.M, Bohrmann, G., Rack, F.R., Torres, M.E., et al., Proc. ODP, Init. Repts., 204: College Station, TX (Ocean Drilling Program), 1–132. doi:10.2973/odp.proc.ir.204.103.2003

Tréhu, A.M., Bohrmann, G., Torres, M.E., and Colwell, F.S. (Eds.), 2006. Proc. ODP, Sci. Results, 204: College Station, TX (Ocean Drilling Program).doi:10.2973/odp.proc.sr.204.2006

Methods & Sampling

DNA extraction and purification

DNA was extracted, in duplicate, from 8 to 20 g of “Mobio” IODP sediment samples previously frozen at -80C using a Powersoil total RNA Isolation Kit with the DNA Elution Accessory Kit (MO-BIO Laboratories, Solana Beach, CA, USA) following the manufacturer protocol but without beads. Approximately 2 grams of sediments

were utilized for each extraction, and DNA pellets from each depth were pooled together. DNA concentrations were measured using a Qubit 2.0 fluorometer with dsDNA High Sensitivity reagents (Invitrogen, Grand Island, NY, USA). The DNA from the extractions that yielded robust 16S rRNA and metagenomic sequences ranged from 3.75-15 ng of DNA per gram of sediments.

16s rRNA gene PCR and amplicon sequencing

The V4 hypervariable region of the 16S rRNA gene was amplified from extracted DNA with barcoded Illumina-specific adapters (38) with technical replication. DNA template (2 ng) was mixed with 5 uL of 10x Taq Mutant reaction buffer, 0.4 uL of Klentaq LA Taq Polymerase (DNA Polymerase Technology, St. Louis, MO, USA), 2 uL of 10 mM dNTP mix (Sigma Aldrich, St. Louis, MO, USA), 2 uL of reverse and forward V4-specific primers F515 and R806 (39) and the remainder DNA-free water to 50 uL (Ambion, Grand Island, NY, USA). PCR was performed on a Bio-Rad C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA) with the following conditions: initial denaturation at 94oC (5 min), followed by 35 cycles of denaturation at 94oC (40 s), primer annealing at 55oC (40 s) and primer extension at 68oC (30 s). The ~400 bp PCR size fragment was confirmed on a 1% agarose gel and replicated samples were pooled. A 50 uL fraction of each pooled sample was purified using a QIAquick PCR Purification Kit (Qiagen, Germantown, MD, USA). Barcoded amplicons for each sample were pooled in equimolar concentrations and sequenced on an Illumina MiSeq (School of Biology, Georgia Institute of Technology), running MiSeq Control Software v.2.4.0.4, using MiSeq reagent kit v2 (500 cycles) with 5% PhiX genomic library control.

Data Processing Description

BCO-DMO Processing:
- Added cruise_id, lat and lon columns

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Data Files

File
16S_accessions.csv (Comma Separated Values (.csv), 2.24 KB) MD5:8f48be1833a480fbd053c5662acb6633 Primary data file for dataset ID 626821

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Parameters

Parameter	Description	Units
cruise_id	cruise identification	unitless
site	site identification	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
BioSample	NCBI BioSample id	unitless
sample_gene	sequenced gene	unitless
Tax_id	NCBI Taxonomy id	unitless
NCBI_accession	NCBI accession number with link	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina MiSeq
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Bio-Rad C1000 Touch Thermocycler
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-204

Website	https://www.bco-dmo.org/deployment/626215
Platform	R/V JOIDES Resolution
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/204PREL.PDF
Start Date	2002-07-07
End Date	2002-09-02
Description	Leg 204 Drilling Gas Hydrates on Hydrate Ridge, Cascadia Continental Margin Sites 1244-1252 7 July-2 September 2002 Cruise report obtained from http://www-odp.tamu.edu/publications/pubs.htm

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Project Information

Functional gene diversity and expression in methane-hydrate bearing deep subsurface sediments (Methane-Hydrate Sediment Omics)

Coverage: Hydrate Ridge, North Pacific, offshore Oregon

Methane is a critical component of the deep subsurface. In shallow marine sediments, anaerobic oxidation of methane (AOM) is coupled to sulfate reduction. However, relatively little is known about which microbial metabolisms are active in deeply buried sediment containing methane hydrates, particularly with regard to alternative electron acceptors that could fuel deep AOM. We propose to determine which microbial population(s) and functional genes are active in the deep biosphere beneath Hydrate Ridge offshore Oregon by sequencing metagenomes and metatranscriptomes from samples drilled on ODP Leg 204 and archived for future molecular analysis. We will analyze gene diversity and expression in six geochemically distinct zones from 2 to 139 meters below the seafloor with the goal of evaluating the relationship between geochemical conditions (i.e. sulfate, iron and manganese availability) and microbial metabolic activity.

Related References:

Tréhu, A.M, Bohrmann, G., Rack, F.R., Torres, M.E., et al., 2003. Proc. ODP, Init. Repts., 204: College Station, TX (Ocean Drilling Program).doi:10.2973/odp.proc.ir.204.2003

Shipboard Scientific Party, 2003. Site 1244. In Tréhu, A.M, Bohrmann, G., Rack, F.R., Torres, M.E., et al., Proc. ODP, Init. Repts., 204: College Station, TX (Ocean Drilling Program), 1–132. doi:10.2973/odp.proc.ir.204.103.2003

Tréhu, A.M., Bohrmann, G., Torres, M.E., and Colwell, F.S. (Eds.), 2006. Proc. ODP, Sci. Results, 204: College Station, TX (Ocean Drilling Program). doi:10.2973/odp.proc.sr.204.2006

This work was supported 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.

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

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