16S rRNA gene sequences of cells in marine methane seep sediments from R/V Atlantis and R/V Western Flyer cruises off the Pacific Northwest, USA in 2010 and 2013

Website: https://www.bco-dmo.org/dataset/662641
Data Type: Cruise Results
Version:
Version Date: 2016-10-25

Project
» Activity-based cell-sorting and enrichment of newly synthesized proteins via amino acid tagging and click chemistry (Cell-sorting and enrichment)

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

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Dataset Description

This dataset contains 16S rRNA gene sequences accession numbers from isolated microbial aggregate from marine methane seep sediments.

These data were published in:

Hatzenpichler, Roland, et al. "Visualizing in situ translational activity for identifying and sorting slow-growing archaeal–bacterial consortia."*Proceedings of the National Academy of Sciences* 113.28 (2016): E4069-E4078. doi:10.1073/pnas.1603757113

Methods & Sampling

Environmental Sampling and Storage (extracted from Hatzenpichler et. al., 2016)

R/V Atlantis cruise AT-15-68:

Sediment sample #3730 was obtained from Hydrate Ridge South methane seep field (Alvin Dive 4635; push-core 16; 44 deg 34.09 N,125 deg 9.14 W; 775 m water depth; sediment horizon 0–6 cm; 4C in-situ temperature) on 7 August 2010. Sediment was stored underargon headspace in a Mylar bag for 5 wk before being transferred toa 1-L glass bottle with a 1.38 bar 100% methane headspace, which was stored at 4C for ~4 years. Seawater and headspace were exchanged at regular intervals to prevent the accumulation of

inhibitory compounds.

MBARI Cruise 2013 "Southern California"

Sediment sample #7142 was collected from Santa Monica Basinon 7 May 2013 (R/V Western Flyer MBARI Cruise 2013; dive 459; push-core 74; 33 deg 47.34 N, 118 deg 40.09 W; 863 m depth; sedimenthorizon 4–6 cm; 4C in situ temperature). The sediment was sealed under argon and stored at 4C. After 40 days of storage, the sediment was suspended in anaerobic natural bottom seawater from the site in an anaerobic chamber (3% H2 in N2) and aliquots were overpressured with 1.5 bar methane. The sediment was kept for 12 months under 1.5 bar 100% methane in natural bottom seawater that was exchanged every 3 mo.

All Sample Handling:

All samples were kept in an ice bath at all times during handling. Artificial seawater (ASW) consisted of 10.9 g of MgCl2·6H2O, 0.2 g of NaHCO3, 0.76 g ofKCl, 25.9 g ofNaCl, 1.47 g of CaCl2·2H2O, 3.98 g of Na2SO4, and 26.73 mg of NH4Cl per liter of ddH2O at pH 7.4. One milliliter of vitamin solution (see medium 141, https://www.dsmz.de) and 1 mL of trace element solution SL- 10 (see https://www.dsmz.de) were also added. Before use, ASW was filtered through a 0.2-µm filter and N2-bubbled for 10 min. ASW was kept on ice during handling. For more information on resuspension and incubation procedures used see Hatzenpichler et. al., 2016.

Data Processing Description

16S rRNA Gene Tag Sequencing (extracted from Hatzenpichler et. al., 2016)

Sediment DNA was extracted using the Power Soil DNA Isolation Kit according to the manufacturer's protocol (MoBio), and diluted DNA from genomeamplified sorted consortia was used directly. The V4 region of the 16S rRNA gene was amplified from each extract using archaeal and bacterial primers 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) (67, 68). Sediment samples were amplified in duplicate. The nonbarcoded primers were used with Q5 Hot Start High-Fidelity 2× Master Mix (New England Biolabs) according to the manufacturer's directions, using annealing conditions of 54 °C for 30 cycles and 58 °C for 32 cycles for sediments and MDAs, respectively. Duplicates of sediment sample amplifications were then pooled. The barcoded 806R primer (CAAGCAGAAGACGGCATACGAGAT XXXXXXXXXXXX AGTCAGTCAG CC GGACTACHVGGGTWTCTAAT) was paired with 515F in a reconditioning reaction (same conditions as above except for five cycles of PCR) to barcode the PCR products. Samples were mixed together in equimolar amounts and purified in bulk through a Qiagen PCR purification kit before submission to Laragen for analysis on an Illumnia MiSeq platform. The resulting paired-end sequence data, 2× 250 bp, was demultiplexed, and sequences with >1 bp mismatch on the 12-bp barcode were removed. The resulting sequences were passed through Illumina's MiSeq Recorder software to assign quality scores to each base call and remove adapter, barcode, and primer sequence.

Information on the fluorescence activiated cell sorted microbial aggregate composed of ANME and SRB, and other probe information can be found in Hatzenpichler et. al., 2016

Data Manager Notes:

- several columns in submitted dataset removed and included in metadata such as publication information.
- converted lat/lon from degrees decimal minutes to decimal degrees.
- added underscores in some text fields
- removed units from data colums. Units can be found in the parameters section.

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Data Files

File
CellSorting.csv (Comma Separated Values (.csv), 19.83 KB) MD5:7fe717888700a47a4e9b8d8c1c8bbb77 Primary data file for dataset ID 662641

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Parameters

Parameter	Description	Units
cruise_id	Cruise Identifier	unitless
dive_id	Dive ID that collected the sample	unitless
collection_date	Date of sample collection	unitless
accession_number	Accesssion number for sequence	unitless
accession_link	Link to accesssion information at NCBI	unitless
organism	Organism description	unitless
lat	latitude	decimal degrees
lon	longitude; west is negative	decimal degrees
water_depth	Water depth at sampling location	meters
sediment_depth	Sample sediment depth	centimeters
collection_source	Description of collection location	unitless

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Deployments

AT15-68

Website	https://www.bco-dmo.org/deployment/58870
Platform	R/V Atlantis
Start Date	2010-07-31
End Date	2010-08-12
Description	Cruise information and original data are available from the NSF R2R data catalog. **Methods & Sampling** 1. Atlantis cruise AT-15-68, Alvin Dive 4635; push-core 16 4. Atlantis cruise AT-18-10, dive J2-593 E4A; push-core 36

MBARI_Southern_California_2013

Website	https://www.bco-dmo.org/deployment/662673
Platform	R/V Western Flyer
Start Date	2013-05-05
End Date	2013-05-22
Description	see MBARI's R/V Western Flyer Expedition Page **Methods & Sampling** R/V Western Flyer MBARI Cruise 2013; dive 463; push-core 43 R/V Western Flyer MBARI Cruise 2013; dive 459; push-core 74

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Project Information

Activity-based cell-sorting and enrichment of newly synthesized proteins via amino acid tagging and click chemistry (Cell-sorting and enrichment)

Website: http://www.darkenergybiosphere.org/outputs-resources/publications/

Coverage: Hydrate Ridge (Oregon) and Santa Monica basin (California)

A fundamental problem faced in deep biosphere research is the low rates at which metabolic activity is occurring in the subsurface. Protein-targeted studies have only rarely been the center of attention, most importantly due to the lack of methodology to visualize protein synthesis within cells and the need for radioactively or stable isotopically labeled substrates to study bulk protein turnover. In response to these limitations, I have recently developed a novel click chemistry-based approach that uses a bioorthogonal non-canonical amino acid to fluorescently track protein synthesis within cells. Within the proposed project I will further develop this method with the goal to establish protocols for the separation and characterization of translationally active cells as well as for the enrichment and identification of newly synthesized proteins. After establishing these techniques using representative pure and co-cultures, I will refine them using enrichments from Hydrate Ridge methane seep sediment. Furthermore, I will study the applicability of other, yet uncharacterized bioorthogonal amino acids to seep sediments as well as >1,700 meters-below-seafloor deep sandstone samples obtained during IODP-expedition 337. This project directly addresses C-DEBI research themes 1, 3, and 4 and will support an early career researcher who has not previously been funded by C-DEBI.

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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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