Geochemistry summary data: single cell isotope incorporation of 1H, 2H, 12C14N, 12C15N, 12C12C, 12C13C ions from Chikyu-337 (IODP 337)

Website: https://www.bco-dmo.org/dataset/712761
Data Type: Cruise Results
Version: 1
Version Date: 2017-08-10

Project
» Determination of deep biosphere cell activity and identity utilizing the state of the art low-biomass, single cell techniques developed at JAMSTEC in their class 10,000 clean room (Deep biosphere cell activity)

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

Contributors	Affiliation	Role
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Abstract
Single cell isotope incorporation of 1H, 2H, 12C14N, 12C15N. 12C12C, 12C13C ions from samples collected on IODP Expedition 337.

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Coverage

Spatial Extent: **Lat**:41.17667 **Lon**:142.2006
Temporal Extent: 2012-07-26 - 2012-09-30

Dataset Description

Single cell isotope incorporation of 1H, 2H, 12C14N, 12C15N. 12C12C, 12C13C ions from samples collected on IODP Expedition 337.

These data are published in:
Trembath-Reichert, E., Morono, Y., Ijiri, A., Hoshino, T., Dawson, K. S., Inagaki, F., & Orphan, V. J. (2017). Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds. Proceedings of the National Academy of Sciences, 114(44), E9206–E9215. doi:10.1073/pnas.1707525114

Acquisition Description

SIP Incubation Preparation
IODP Expedition 337 operations commenced July 26 and continued through September 30, 2012 on the D/V Chikyu. Utilizing riser drilling, a sedimentary sequence was recovered down to 2466 m below seafloor (mbsf) at Hole C0020A (41 10' 36" N, 142 12' 02" E) in 1180 m water depth off the Shimokita Peninsula. The drilled sequence transitioned from open marine (youngest; late Pliocene, ~5 Ma) to terrestrial (oldest; late Oligocene, ~30 Ma) with depth. Models for maximum temperature reached by Expedition 337 coring report 63.7 degrees C. Shipboard sedimentological, geochemical, and microbiological data and methods are available through IODP publications. Additional coal petrography is available in Gross et al. A total of 52 incubation amendment conditions were prepared onboard to interrogate a range of potential deep-biosphere metabolic strategies, and then incubated back in the lab at temperatures approximating that measured in situ. In this study, incubations from shale (Core 8L4; 1606 mbsf; 37C incubation temperature), coal (Core 15R3; 1921 mbsf; 45C incubation temperature), and mixed (homogenized mixture from multiple cores 19R1, 19R5, 19R7, 20R3, 23R6, 23R8, 24R3, 25R1, 25R2, and 25R3; 1950-1999 mbsf; 45C incubation temperature) with methanol and methylamine substrate additions were analyzed. Age estimates of these samples are early to middle Miocene. In situ temperatures ranged from 38C to 48C at these

sample depths with pressures ~30 MPa. Two coal beds were included in these incubations: a shallower coal-only sample deposited under more marine-influenced conditions (~1921 mbsf, core 15R3) and a deeper coal bed deposited under more limnic conditions that was included in the mixed lithology sample (~2000 mbsf, cores 24R3 and 25R1). Cores used for incubations were prepared by removal of outer drill-fluid-contaminated layers by sterile ceramic knife as soon as possible after core recovery and stored at 4C until incubation preparation, while maintaining an anaerobic atmosphere during the entire process. For preparation of the SIP incubations, the interior portion of the core was manually crushed into cm-sized pieces under sterile, anaerobic conditions and distributed evenly into sterile 50 ml glass vials with butyl rubber stoppers and screw caps (Nichidenrika-Glass Co. Ltd.). Vials were flushed with argon and pressurized to 1 atm argon headspace. Sterile C-, N-, and S-free media (1% PBS, 30 g/L NaCl, 12 g/L MgCl2, and 3 g/L KCl) was prepared anaerobically with deuterated water (20 at. % 2H2O). 20 at. % 2H2O was selected as the highest level of enrichment with little to no effect on the activity of microorganisms in pure culture. Time point 1, time point 2, and autoclaved treatments were prepared for each substrate condition. Time point 1 incubations lasted for six months, while time point 2 and autoclaved treatments were maintained at the in situ incubation temperature for 2.5 years. Due to low levels of activity ascertained from geochemical measurements, all NanoSIMS analyses were conducted on time point 2 and autoclaved samples. Amendments and incubation conditions for the methyl-substrate subset analyzed in this study are provided in this dataset. Equimolar amounts of substrate (30 umol C, 1.5 mM final; 3 umol N, 0.15 mM final) were added across incubation conditions at 50 at. % (Cambridge Isotopes). Hydrogen was added as 5 mL 100% H2 overpressure to incubations (~15% H2 headspace). A full list of the additional incubation conditions prepared onboard are listed in cruise Methods. Alkalinity (34.39 – 9.68 mM) ammonium (2.80 – 1.83 mM) concentrations from formation fluid samples collected onboard exceed concentrations of C and N amendments. Concentrations of methylamine (0.05 mM) and methanol (1 mM) measured from lignite coal also suggest our substrate additions were environmentally relevant. After 30 months of incubation (March 2014) all treatments were sampled for geochemical analyses prior to preparation for NanoSIMS. 3 ml of headspace gas was removed to a vial filled with 0.1 M NaOH for methane analysis. About 1 ml of liquid was filtered through a 0.1 um 13 mm Whatman Polycarbonate Nuclepore Track-Etched Membrane (110405) for DIC analysis. See Supplemental Methods of Trembath-Reichert et al. for detailed description of methane and DIC analyses.

Sample preparation for NanoSIMS analysis

To overcome technical challenges for NanoSIMS analysis of low biomass samples, cell separation and fluorescence-activated cell sorting (FACS) were used to directly concentrate cells in a small analysis area, ~1 to 0.5 sq. mm. NanoSIMS samples were prepared from paraformaldehyde (PFA)-fixed cell separates after 894 days of incubation. Cell preservation, separation, enumeration, and FACS were all conducted in the clean booth and clean room facilities at Kochi Institute for Core Sample Research, JAMSTEC. Half of the solid and half of

the liquid portion of each sample were fixed overnight in a solution of 2% paraformaldehyde (PFA), 3 × phosphate buffered saline (PBS). Samples were then subjected to two washes, incubating in 3 × PBS for 6 hrs and then 2 hrs, after each wash respectively. Samples were centrifuged (3500 × *g*) and supernatant was decanted after each wash. PFA-fixed samples were stored in 50 % ethanol : 3 × PBS. The other half of the sample was preserved in glyTE (70% glycerol, 100mM Tris, 10mM EDTA; Bigelow Single Cell Genomics Center preservation protocol) and frozen by cell alive system (CAS) and stored at -80C. 1 ml liquid and ~1 g sediment chips were subsampled by pipet and sterile cell culture loop, respectively, from the PFA-fixed sample. Cell separation, microscopy, and sorting procedures followed Morono et al., with the following modifications: 1) samples were sonicated (Bioruptor UCD-250, COSMO BIO) in an ice bath for 20 cycles of 30 sec 200 W, 30 sec off, and 2) samples were incubated in hydrofluoric acid post initial sonication, rather than after first density gradient separation. Cell detection limit was determined by no-sample added controls run in parallel with samples. Cells were stained with SYBR Green I (1:40 dilution of SYBR Green in Tris (10 mM) –EDTA (1mM) (TE) and sorted following the flow cytometry protocol of Morono et al. Sorted cells were concentrated directly from the sorter onto NanoSIMS compatible 0.2 um polycarbonate filters coated with indium tin oxide (ITO) as described in Morono et al. and Inagaki et al. ITO coating on polycarbonate membranes (Isopore GTBP02500 Millipore) was prepared by sputtering deposition technique at Astellatech Co. Ltd. (Kanagawa, Japan). Scanning electron microscopy (SEM) of the filters was done on a Zeiss 1550 VP Field Emission Scanning Electron Microscope at the GPS Division Analytical Facility at Caltech and SYBR stained cells were imaged with a BX51 epifluorescence microscope (Olympus, Tokyo, Japan) using 20× (UPlanFL N) dry, 60× (PlanApo N), and 100× (UPlanFL N) oil immersion objectives.

Processing Description

NanoSIMS instrument tuning and analysis:
Cell targets were identified (by SYBR stain) and marked on NanoSIMS membranes with a laser dissection microscope (LMD6000; Leica Microsystems) for ease of rediscovery on the NanoSIMS. Samples were analyzed by raster ion imaging with a CAMECA NanoSIMS 50L at the Caltech Microanalysis Center in the Division of Geological and Planetary Sciences. A focused primary Cs+ beam of ~ 1 pA was used for sample collection, with rasters of 256 × 256 or 512 × 512 pixels. 1H (EM#1), 2H (EM#2), 12C2 (EM#3), 13C12C (EM#4), 12C14N (EM#5), and 12C15N (EM#6) were measured simultaneously.Collection began after a pre-sputtering of equal intensity to one collection frame (~45 min).

Data Processing:
Recorded images and data were processed using Look@NanoSIMS software. Images were deadtime-corrected and individual ion image frames were merged and aligned using the

12C14N ion image to correct for drift during acquisition. Cell-based regions of interest (ROIs) were determined by "interactive thresholding" with the 12C14N ion image. Final ion images and counts per ROI were calculated by summation of ion counts for each pixel over all scans. Outputs for ROI size were used to compute cell diameters. We also confirmed that cell ROIs had a total C to total N ratio that was distinct from the background correction ROIs or coal to ensure drawn ROIs only included biomass targets.

Further information about the data output can be found in the LANS Manual (PDF).

BCO-DMO Data Processing:
- modified parameter names to conform with BCO-DMO naming conventions

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Related Publications

Trembath-Reichert, E., Morono, Y., Ijiri, A., Hoshino, T., Dawson, K. S., Inagaki, F., & Orphan, V. J. (2017). Methyl-compound use and slow growth characterize microbial life in 2-km-deep subseafloor coal and shale beds. Proceedings of the National Academy of Sciences, 114(44), E9206–E9215. doi:10.1073/pnas.1707525114

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Parameters

Parameter	Description	Units
Order	Count of samples	unitless
Sample	Sample name/identifier	unitless
Horizon	IODP core name incubation originates from	unitless
Condition	Number code for Condition notes	unitless
Autoclaved	Autoclaved? Yes (Y) or No (N)	unitless
Amendment	Amendments (what was added to the incubation)	unitless
cells_per_ml	Number of cells per milliliter of sample	number/mL
cells_per_cc_rock	Number of cells per cubic centimeter of sample	number/(cm^3)
cells_per_g_rock	Number of cells per gram of rock	number/g
Del_13C_DIC	delta 13C DIC	per mil
mM_DIC	Concentration of DIC in mM	milliMolar (mM)

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Instruments

Dataset-specific Instrument Name	BX51 epifluorescence microscope
Generic Instrument Name	Microscope-Fluorescence
Dataset-specific Description	SYBR stained cells were imaged with a BX51 epifluorescence microscope (Olympus, Tokyo, Japan).
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	LMD6000
Generic Instrument Name	Microscope-Optical
Dataset-specific Description	Cell targets were identified (by SYBR stain) and marked on NanoSIMS membranes with a laser dissection microscope (LMD6000; Leica Microsystems) for ease of rediscovery on the NanoSIMS.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	Zeiss 1550 VP Field Emission Scanning Electron Microscope
Generic Instrument Name	Scanning Electron Microscope
Dataset-specific Description	Scanning electron microscopy (SEM) of the filters was done on a Zeiss 1550 VP Field Emission Scanning Electron Microscope at the GPS Division Analytical Facility at Caltech.
Generic Instrument Description	Scanning electron microscope

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Deployments

Chikyu-337

Website	https://www.bco-dmo.org/deployment/712655
Platform	Chikyu
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/337PR.PDF
Start Date	2012-07-26
End Date	2013-09-30
Description	Integrated Ocean Drilling Program Expedition 337 Deep Coalbed Biosphere off Shimokita Microbial processes and hydrocarbon system associated with deeply buried coalbed in the ocean 26 July–30 September 2012 More information from IODP: http://publications.iodp.org/preliminary_report/337/index.html

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Project Information

Determination of deep biosphere cell activity and identity utilizing the state of the art low-biomass, single cell techniques developed at JAMSTEC in their class 10,000 clean room (Deep biosphere cell activity)

Website: http://www.darkenergybiosphere.org/award/determination-of-deep-biosphere-cell-activity-and-identity-utilizing-the-state-of-the-art-low-biomass-single-cell-techniques-developed-at-jamstec-in-their-class-10000-clean-room/

Coverage: Hole C0020A (41°10′36″N, 142°12′02″E) Shimokita Peninsula.

IODP Expedition 337 set the record for deepest marine scientific drilling down to 2.4 kmbsf. This cruise also had the unique opportunity to retrieve deep cores from the Shimokita coal bed system in Japan with the aseptic and anaerobic conditions necessary to look for deep life. Onboard scientists prepared nearly 1,700 microbiology samples shared among five different countries to study life in the deep biosphere. Samples spanned over 1km in sampling depths and include representatives of shale, sandstone, and coal lithologies. Findings from previous IODP and deep mine expeditions suggest the genetic potential for methylotrophy in the deep subsurface, but it has yet to be observed in incubations. A subset of Expedition 337 anoxic incubations were prepared with a range of 13C-methyl substrates (methane, methylamine, and methanol) and maintained near in situ temperatures. To observe 13C methyl compound metabolism over time, we monitored the δ13C of the dissolved inorganic carbon and methane

(by-products of methyl compound metabolism) over a period of 1.5 years. Our geochemical evidence suggests that the coal horizon incubated with 13C-methylamine showed the highest activity of all methyl incubations. Therefore, there are not only cells in the deeply buried terrigenous coal bed at Shimokita, but a microbial community that can be activated by methylotrophic compounds. Incubations showing the highest geochemical activity were prepared at the JAMSTEC Kochi Core Center for nanoSIMS analysis in March of 2015, and will be analyzed at Caltech in the coming months. This will allow us to observe if cells also incorporated the labeled methyl compounds into their body mass and provide another line of evidence that these substrates were used by the deep coalbed microbial community.

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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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