Diversity of archaeal intact polar lipids (IPLs) and changes in relative abundance of bacterial dietherglycerol lipids (DEGs) within the selected 11 samples at different depths

Website: https://www.bco-dmo.org/dataset/794671
Data Type: Other Field Results
Version: 0
Version Date: 2020-02-28

Project
» Collaborative Research: Delineating The Microbial Diversity and Cross-domain Interactions in The Uncharted Subseafloor Lower Crust Using Meta-omics and Culturing Approaches (Subseafloor Lower Crust Microbiology)

Program
» International Ocean Discovery Program (IODP)

Contributors	Affiliation	Role
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Dataset Description

Diversity of archaeal intact polar lipids (IPLs) and changes in relative abundance of bacterial dietherglycerol lipids (DEGs) within the selected 11 samples at different depths (meters below sea floor; mbsf).

Methods & Sampling

Crushed core samples stored in falcon tubes at -80 °C were first milled for 10 min to a fine powder and subsequently extracted with a modified Bligh and Dyer method after Sturt et al. (2004). Prior to milling and extraction of each sample a procedure blank was performed. First a milling blank was performed using combusted sea sand (fired at 450 °C for 5 hrs) to clean the mill and to limit cross-contamination of samples. Subsequently, this sea sand was then transferred to geo-cleaned (rinsed three times with a mixture of methanol, MeOH and dichloromethane, DCM) Teflon® containers used for extraction of the samples and solvent-extracted in the same manner as the samples. For this, 100 ng of an internal standard (C46 GTGT) and ca. 50 mL of a solvent mixture of DCM:MeOH:buffer (2:1:0.8, v/v) was added to the sample in the Teflon® container and ultrasonicated for 10 mins using a geo-cleaned ultrasonic stick. After ultrasonication, the samples were centrifuged (1750 rpm at 10 min) and the supernatant was transferred to a fired separatory funnel. The samples were extracted in four steps, for the first two steps a phosphate buffer (K2HPO4, 50 mM at pH 7.4) was used, in the second step the phosphate buffer was replaced by 5 was used, in the second step the phosphate buffer was replaced by 5 % trichloroacetic acid (50 g L-1 at pH 2), and in the last step only DCM:MeOH (9:1, v/v) was used. Equal amounts of DCM and deionized MilliQ water were added to the extract collected in the separatory funnel, the mixture was shaken, and the organic phase was collected as the total lipid extract (TLE) and blown to dryness under a gentle stream of nitrogen. An aliquot of the TLE was analyzed

via ultra-high-pressure liquid chromatography (UHPLC) coupled to mass spectrometry (MS) on a Dionex Ultimate 3000RS UHPLC connected to an ABSciEX QTRAP4500 Triple Quadrupole/Ion Trap MS (UHPLC-Triple Quad-MS) via a Turbolon electrospray ion source (ESI). Separation of compounds was achieved on a Waters Acquity BEH C18 column (1.7 µm, 2.1x150 mm) equipped with a guard column of the same material following the protocol described in Klein et al. (2015). Compounds of interest were screened for by using multiple reaction monitoring (MRM) and selected ion monitoring (SIM) techniques after Klein et al. (2015). Concentrations of lipids were determined relative to the internal C46 GTGT standard and were corrected for individual response factors using commercially available standards (diC16-DEG, archaeol) and isolated standards from cultures (GDGT-0, 1G-AR, 2G522AR, 1G-GDGT-0, 2G-GDGT-0). The presence of crenarchaeol was confirmed by core GDGT analysis after Becker et al. (2013). Briefly an aliquot of the TLE was analysed on Dionex Ultimate 3000RS UHPLC connected to a Bruker maXis ultra-high resolution quadrupole time-of-flight mass spectrometer, equipped with an APCI II source. Compounds were separated using two aquity BEH HILIC amide columns (1.7 µm, 2.1x300 mm) in tandem maintained at 50 °C, and n-hexane as eluent A and n528 hexane:isopropanol, 90:10, v:v as eluent B (REF). Drilling mud and extraction blank contamination controls were also run for lipid biomarker analyses.

Data Processing Description

BCO-DMO Processing Notes:
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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Related Publications

Becker, K. W., Lipp, J. S., Zhu, C., Liu, X.-L., & Hinrichs, K.-U. (2013). An improved method for the analysis of archaeal and bacterial ether core lipids. Organic Geochemistry, 61, 34–44. doi:10.1016/j.orggeochem.2013.05.007
Methods

Klein, A. T., Yagnik, G. B., Hohenstein, J. D., Ji, Z., Zi, J., Reichert, M. D., … Lee, Y. J. (2015). Investigation of the Chemical Interface in the Soybean–Aphid and Rice–Bacteria Interactions Using MALDI-Mass Spectrometry Imaging. Analytical Chemistry, 87(10), 5294–5301. doi:10.1021/acs.analchem.5b00459
Methods

Sturt, H. F., Summons, R. E., Smith, K., Elvert, M., & Hinrichs, K.-U. (2004). Intact polar membrane lipids in prokaryotes and sediments deciphered by high-performance liquid chromatography/electrospray ionization multistage mass spectrometry—new biomarkers for biogeochemistry and microbial ecology. Rapid Communications in Mass Spectrometry, 18(6), 617–628. doi:10.1002/rcm.1378
Methods

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Parameters

Parameter	Description	Units
Sample_ID	Sample ID	percent (%)
Depth	depth below seafloor	meters (m)
IPL	Intact Polar lipid concentration	picograms per gram (pg/g)
G1_GDGT	mono glycerol dialkyl glycerol tetraether	percent (%)
G2_GDGT	diglycosidic glycerol dialkyl glycerol tetraether	percent (%)
G1_AR	mono archaeol	percent (%)
G2_AR	diglycosidic archaeol	percent (%)
DEG	dietherglycerol lipids	picograms per gram (pg/g)
C28_C30	carbon chain lengths from C28-C30	percent (%)
C31_C33	carbon chain lengths from C31-C33	percent (%)
C34_C36	carbon chain lengths from C34-C36	percent (%)
C37_C39	carbon chain lengths from C34-C36	percent (%)

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Instruments

Dataset-specific Instrument Name	ultra-high-pressure liquid chromatography (UHPLC)
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	All membrane lipid analyses were performed at MARUM (Bremen, Germany) with an ultra-high-pressure liquid chromatography (UHPLC) coupled to mass spectrometry (MS) on a Dionex Ultimate 3000RS UHPLC connected to an ABSciEX QTRAP4500 Triple Quadrupole/Ion Trap MS (UHPLC-Triple Quad-MS) via a Turbolon electrospray ion source (ESI).
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	ABSciEX QTRAP4500 Triple Quadrupole/Ion Trap MS (UHPLC-Triple Quad-MS)
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	ll membrane lipid analyses were performed at MARUM (Bremen, Germany) with an ultra-high-pressure liquid chromatography (UHPLC) coupled to mass spectrometry (MS) on a Dionex Ultimate 3000RS UHPLC connected to an ABSciEX QTRAP4500 Triple Quadrupole/Ion Trap MS (UHPLC-Triple Quad-MS) via a Turbolon electrospray ion source (ESI).
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

Collaborative Research: Delineating The Microbial Diversity and Cross-domain Interactions in The Uncharted Subseafloor Lower Crust Using Meta-omics and Culturing Approaches (Subseafloor Lower Crust Microbiology)

Coverage: SW Indian Ridge, Indian Ocean

NSF abstract:

The lower ocean crust has remained largely unexplored and represents one of the last frontiers for biological exploration on Earth. Preliminary data indicate an active subsurface biosphere in samples of the lower oceanic crust collected from Atlantis Bank in the SW Indian Ocean as deep as 790 m below the seafloor. Even if life exists in only a fraction of the habitable volume where temperatures permit and fluid flow can deliver carbon and energy sources, an active lower oceanic crust biosphere would have implications for deep carbon budgets and yield insights into microbiota that may have existed on early Earth. This is all of great interest to other research disciplines, educators, and students alike. A K-12 education program will capitalize on groundwork laid by outreach collaborator, A. Martinez, a 7th grade teacher in Eagle Pass, TX, who sailed as outreach expert on Drilling Expedition 360. Martinez works at a Title 1 school with ~98% Hispanic and ~2% Native American students and a high number of English Language Learners and migrants. Annual school visits occur during

which the project investigators present hands on-activities introducing students to microbiology, and talks on marine microbiology, the project, and how to pursue science related careers. In addition, monthly Skype meetings with students and PIs update them on project progress. Students travel to the University of Texas Marine Science Institute annually, where they get a campus tour and a 3-hour cruise on the R/V Katy, during which they learn about and help with different oceanographic sampling approaches. The project partially supports two graduate students, a Woods Hole undergraduate summer student, the participation of multiple Texas A+M undergraduate students, and 3 principal investigators at two institutions, including one early career researcher who has not previously received NSF support of his own.

Given the dearth of knowledge of the lower oceanic crust, this project is poised to transform our understanding of life in this vast environment. The project assesses metabolic functions within all three domains of life in this crustal biosphere, with a focus on nutrient cycling and evaluation of connections to other deep marine microbial habitats. The lower ocean crust represents a potentially vast biosphere whose microbial constituents and the biogeochemical cycles they mediate are likely linked to deep ocean processes through faulting and subsurface fluid flow. Atlantis Bank represents a tectonic window that exposes lower oceanic crust directly at the seafloor. This enables seafloor drilling and research on an environment that can transform our understanding of connections between the deep subseafloor biosphere and the rest of the ocean. Preliminary analysis of recovered rocks from Expedition 360 suggests the interaction of seawater with the lower oceanic crust creates varied geochemical conditions capable of supporting diverse microbial life by providing nutrients and chemical energy. This project is the first interdisciplinary investigation of the microbiology of all 3 domains of life in basement samples that combines diversity and "meta-omics" analyses, analysis of nutrient addition experiments, high-throughput culturing and physiological analyses of isolates, including evaluation of their ability to utilize specific carbon sources, Raman spectroscopy, and lipid biomarker analyses. Comparative genomics are used to compare genes and pathways relevant to carbon cycling in these samples to data from published studies of other deep-sea environments. The collected samples present a rare and time-sensitive opportunity to gain detailed insights into microbial life, available carbon and energy sources for this life, and of dispersal of microbiota and connections in biogeochemical processes between the lower oceanic crust and the overlying aphotic water column.

About the study area:

The International Ocean Discovery Program (IODP) Expedition 360 explored the lower crust at Atlantis Bank, a 12 Ma oceanic core complex on the ultraslow-spreading SW Indian Ridge. This oceanic core complex represents a tectonic window that exposes lower oceanic crust and mantle directly at the seafloor, and the expedition provided an unprecedented opportunity to access this habitat in the Indian Ocean.

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Program Information

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-1658031

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