Carbon isotopic compositions and fractionation factors of M. jannaschii in high and low hydrogen (H2) environments

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Project
» Bioenergetic influences upon carbon flow in alkaliphilic sulfate-reducing microbial populations with relevance to the subsurface biosphere at the Lost City Hydrothermal Field (Carbon flow through SRB)

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

Contributors	Affiliation	Role
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Abstract
Carbon isotopic compositions and fractionation factors of amino acids and squalenoid lipids in Methanocaldococcus jannaschii in high and low hydrogen (H2) environments.

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Coverage

Spatial Extent: **Lat**:30.11667 **Lon**:-42.11667

Dataset Description

Carbon isotopic compositions and fractionation factors of amino acids and squalenoid lipids in Methanocaldococcus jannaschii in high and low hydrogen (H2) environments.

Methods & Sampling

Isolation and isotopic analysis of squalenoids:
Cell pellets were freeze-dried overnight, ground with a clean spatula, and extracted three times by sonication in a centrifuge tube filled with 50 mL of 3:1 dichloromethane:methanol (DCM:MeOH). All glassware was combusted overnight at 500°C to remove organics prior to use. After sonication, the extracts were spun in a centrifuge at 125 g for 15 min and the supernatant was decanted to a separate vial. All extracts were combined and the solvent was evaporated to dryness in a rotary evaporator. A maximum of 2 mL of 9:1 DCM:MeOH was added to dissolve the total extract that was then passed over Na2SO4 to remove water. The water-free extract was then separated into different fractions over SepraTM NH2 bulk-packing (P/N

1001711653 572122 – U) silica column by eluting with solvents of increasing polarity (F1 = 5 mL of hexane, F2 = 6 mL of 3:1 hexane:DCM, F3 = 7 mL of 9:1 DCM:acetone, F4 = 8 mL of 4% formic acid in DCM). The apolar fraction (F1) was dried under N2, then re-dissolved in 50 µL of hexane for identification.

The lipids in the apolar fraction were identified and quantified using an Agilent Technologies 5975 inert XL Mass Selective Detector after separation on an Agilent J&W GC HP-5MS UI capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, P/N 19091S – 433UIE) using He as the carrier gas. Samples were injected in pulse splitless mode. The GC oven was from an initial temperature of 70°C, then heated to 150°C at 15°C per min, then to 300°C at 5°C per min. Peaks were quantified by comparison to a 5-point standard curve of a C7-C30 alkane series (P/N 49451 – U, Sigma Aldrich). The isotopic composition of biomarkers in the apolar fraction was determined on a Thermo Scientific Gas Chromatograph-IsolinkII-Isotope Ratio Mass Spectrometer (GC-IsolinkII-IRMS) equipped with an Agilent DB-5 fused silica column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with He as the carrier gas.

Isolation, characterization, and isotopic analysis of amino acids:
Pelleted cells were hydrolyzed with 6 M HCl (Ultrapure grade) with 1% of 11 mM ascorbic acid under N2 at 110°C for 20 h (Henrichs, 1991). After cooling, hydrolyzed amino acids were spiked with internal standard norvaline and derivatized with acidified isopropanol and acetyl chloride for 1 h at 110°C (Silfer et al., 1991). The samples then reacted at 110°C for 1 h on a hot plate. They were then esterified with trifluoroacetic anhydride (TFAA) for 10 min at 110°C for 10 min. The resulting derivatives were dissolved in dichloromethane. The isotopic signatures of derivatized amino acids were determined by GC-IsolinkII-IRMS

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Data Files

File
hilowH2_isotopes.csv (Comma Separated Values (.csv), 1.29 KB) MD5:fb5729658947e3a17c5dfec4fceea811
Primary data file for dataset ID 812240

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Related Publications

Nguyen, T. B., Topçuoğlu, B. D., Holden, J. F., LaRowe, D. E., & Lang, S. Q. (2020). Lower hydrogen flux leads to larger carbon isotopic fractionation of methane and biomarkers during hydrogenotrophic methanogenesis. Geochimica et Cosmochimica Acta, 271, 212–226. doi:10.1016/j.gca.2019.11.015
Results

Topçuoğlu, B. D., Meydan, C., Nguyen, T. B., Lang, S. Q., & Holden, J. F. (2019). Growth Kinetics, Carbon Isotope Fractionation, and Gene Expression in the Hyperthermophile Methanocaldococcus jannaschii during Hydrogen-Limited Growth and Interspecies Hydrogen Transfer. Applied and Environmental Microbiology, 85(9). doi:10.1128/aem.00180-19 https://doi.org/10.1128/AEM.00180-19
Methods

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Parameters

Parameter	Description	Units
Experiment	Experiment description: either high (abundant) or low (limited) hydrogen	unitless
Culture_ID	culture identifier	unitless
d13C_ppt_Ala	13C isotopic ratio of alanine	parts per thousand (ppt)

d13C_ppt_Gly	13C isotopic ratio of glycine	parts per thousand (ppt)
d13C_ppt_Thr	13C isotopic ratio of threonine	parts per thousand (ppt)
d13C_ppt_Ser	13C isotopic ratio of serine	parts per thousand (ppt)
d13C_ppt_Val	13C isotopic ratio of valine	parts per thousand (ppt)
d13C_ppt_Leu	13C isotopic ratio of leucine	parts per thousand (ppt)
d13C_ppt_Iso	13C isotopic ratio of isoleucine	parts per thousand (ppt)
d13C_ppt_Pro	13C isotopic ratio of proline	parts per thousand (ppt)
d13C_ppt_Glu	13C isotopic ratio of glu	parts per thousand (ppt)
d13C_ppt_Phe	13C isotopic ratio of phenylalanine	parts per thousand (ppt)
Weighted_Avg_d13C_THAA_ppt	Weighted isotopic ratio of each amino acid as a toal	parts per thousand (ppt)
d13C_ppt_Sq_3	13C isotopic ratio of squalenoid with three double bonds	parts per thousand (ppt)
d13C_ppt_Sq_4	13C isotopic ratio of squalenoid with four double bonds	parts per thousand (ppt)
d13C_ppt_Sq_5	13C isotopic ratio of squalenoid with five double bonds	parts per thousand (ppt)
d13C_ppt_Sq_6	13C isotopic ratio of squalene	parts per thousand (ppt)
Weighted_Avg_ppt_d13C_Sq	weighted average of the isotopic composition of squalenoids	parts per thousand (ppt)
DIC_mM_To	Concentration of dissolved inorganic carbon at the start of the experiment	milliMolar (mM)
DIC_mM_Tf	Concentration of dissolved inorganic carbon at the end of the experiment	milliMolar (mM)
TFAA_uM_To	Concentration of total free amino acids at the start of the experiment	microMolar (uM)
TFAA_uM_Tf	Concentration of total free amino acids at the end of the experiment	microMolar (uM)
THAA_uM_To	Concentration of total hydrolizable amino acids at the start of the experiment	microMolar (uM)
THAA_uM_Tf	Concentration of total hydrolizable amino acids at the end of the experiment	microMolar (uM)
Fractionation_factor_ppt_eCO2_CH4	Fractionation factor between CO2-and methane	parts per thousand (ppt)
Fractionation_factor_ppt_eCO2_B	Fractionation factor between CO2-and biomass	parts per thousand (ppt)
Fractionation_factor_ppt_eCO2_AA	Fractionation factor between CO2-and amino acids	parts per thousand (ppt)
Fractionation_factor_ppt_eCO2_squalenoids	Fractionation factor between CO2-and squalenoids	parts per thousand (ppt)

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Instruments

Dataset-specific Instrument Name	Gas Chromatograph Mass Spectrometer
Generic Instrument Name	Gas Chromatograph Mass Spectrometer
Dataset-specific Description	For or identification and abundance of carbon isotopes. Agilent Technologies 5975 inert XL Mass Selective Detector after separation on an Agilent J&W GC HP-5MS UI capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness, P/N 19091S – 433UIE)
Generic Instrument Description	Instruments separating gases, volatile substances or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay by a mass spectrometer.

Dataset-specific Instrument Name	Thermo Scientific Trace 1310 Gas Chromatograph-IsolinkII-Delta V Isotope Ratio Mass Spectrometer
Generic Instrument Name	Gas Chromatograph Mass Spectrometer
Dataset-specific Description	For measuring isotopic composition of biomarkers, including amino acid and lipids: Thermo Scientific Trace 1310 Gas Chromatograph-IsolinkII-Delta V Isotope Ratio Mass Spectrometer equipped with an Agilent DB-5 fused silica column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and a Gerstel CIS – 6 inlet.
Generic Instrument Description	Instruments separating gases, volatile substances or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay by a mass spectrometer.

Dataset-specific Instrument Name	Thermo Scientific GasBench- Delta V Isotope Radio Mass Spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Dataset-specific Description	For measuring isotopic composition of dissolved inorganic carbon.
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

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Project Information

Bioenergetic influences upon carbon flow in alkaliphilic sulfate-reducing microbial populations with relevance to the subsurface biosphere at the Lost City Hydrothermal Field (Carbon flow through SRB)

Coverage: Atlantis Massif, 30 8'N, 42 8'W

Project description from C-DEBI:
The microbial biosphere in serpentinizing subseafloor rocks is globally significant. Tantalizing evidence from studies of the Lost City Hydrothermal Field and continental ophiolites indicates that hydrogendriven microbial metabolisms prevails under the highly reducing, high pH conditions that characterize these environments. Interest in these processes is evident from an upcoming cruise to the Atlantis Massif in Fall 2015 to obtain drill cores in the vicinity of the Lost City Hydrothermal Field (IODP Expedition #357; both PIs were proponents of the IODP proposal and have applied as shipboard scientists). The PIs and colleagues have made headway over the last decade in identifying the key organisms and metabolisms present at the LCHF, and in constraining the sources and fates of carbon compounds. The linkages between geology and biology remain enigmatic, however, because of the precipitation of inorganic carbon at high pHs and overlapping biogenic and abiogenic carbon sources. We propose here to investigate the influence of free energy availability by sulfate reduction in resource utilization and carbon flow by model alkaliphilic prokaryotes. The laboratory approach using a model system will inform shipboard experiments with fresh samples from the AM, and the potential characterization of new organisms from serpentinizing terrains.

This project was funded 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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0939564

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