16S rRNA gene sequences of bacteria including members of the Arctic96BD-19/SUP05 Clade of marine bacteria.

Website: https://www.bco-dmo.org/dataset/626596
Version: final
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Project
» Mixotrophic bacteria and the cryptic marine sulfur cycle: Mechanisms of carbon assimilation and sulfur oxidation in the Arctic96BD-19 GSO clade (Sulfur Oxidizers)

Contributors	Affiliation	Role
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Coverage

Spatial Extent: **N**:47.687 **E**:-122.402 **S**:45.8202 **W**:-129.756
Temporal Extent: 2009-08-11 - 2011-11-11

Dataset Description

16S rRNA gene sequences of bacteria cultured in by High Throughput Cultivation, including members of the Arctic96BD-19/SUP05 Clade of marine bacteria.

These data are reported and discussed in Marshall and Morris, 2012. (doi: 10.1038/ismej.2012.78)

Methods & Sampling

Members of the SUP05/Arctic96BD-19 clade of gamma proteobacterial sulfur oxidizers (GSOs) were cultured using a high-throughput dilution to extinction culturing approach modified from Connon and Giovannoni (2002). Culturing experiments were conducted with surface water collected from the Puget Sound main basin (47° 41.24' N, 122° 24.14' W) in November 2009 and from the deep chlorophyll maximum (DCM) (45 m) in the North Pacific gyre near Axial seamount in August 2011.

Data Processing Description

Culture media was prepared by pre-filtering seawater through a 0.8 µm polyethersulfone filter (Supor-200, Pall Corp, Ann Arbor, MI) and by sterilizing the filtrate using a 30 kD biomax polyethersulfone tangential flow filtration (TFF) cartridge (Millipore, Billerica, MA). TFF filter-sterilized seawater media was collected in autoclaved polycarbonate bottles and stored at in situ temperatures. Matching whole water samples were diluted (3-5 cells

ml-1) in TFF filter-sterilized seawater media and added to each well of an acid washed (10% HCL) 96 well Teflon plate (Sonomatesting, Forestville, CA).

Each experiment consisted of 576 cultures divided into two treatments. One treatment contained filter sterilized seawater media (unamended) and one contained seawater media amended with a natural source of organic carbon (lysate). Vitamins B1, B6, B7, and B12 were added to the North Pacific gyre lysate treatment at a final concentration of 10 nM each. Plates were incubated in the dark at in situ temperatures (Puget Sound, 13 °C and North Pacific, 10 °C) and screened for growth on an Easyflow Guava flowcytometer equipped with a 96 well plate reader (Millipore, Billerica, MA). Cultures were checked for growth by transferring 150 µL of culture to a new plate and by staining the cells with Syber Green I (Invitrogen, Carlsbad, CA) diluted in TRIS buffer and at a final concentration of 1/2000, as previously described (Stingl et al., 2007).

Taxonomic assignments were determined for bacterial cultures that were positive for growth by extracting and amplifying the 16S rRNA gene. DNA from 200 µl of culture was extracted using a DNeasy Blood and Tissue Kit (QIAGEN, Germantown, MD, USA). 16S rRNA genes were amplified using a semi-nested PCR reaction with Taq polymerase (Fermentas, Hannover, MD, USA) and bacterial primers. Amplifications were performed in a C1000 thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following conditions: 35 cycles with 8F and 1492R primers followed by 38 cycles with 8F and 519R primers. The same conditions were used for each PCR reaction; denaturation at 94 °C for 30 s., annealing at 55 °C for one min, elongation at 72 °C for two min, and a final elongation step at 72°C for 10 min Amplicons were sequenced at the High-Throughput Genomics Unit (University of Washington, Seattle, WA, USA). Taxonomic assignments were determined using the Bayesian method of Wang et al., (2007) and a database augmented with sequences from marine environmental clades as previously described (Iverson et al., 2012).

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Data Files

File
16S_rRNA_seq.csv (Comma Separated Values (.csv), 19.08 KB) MD5:0d358083f9bd0dcd7922ac8d30a2404b Primary data file for dataset ID 626596

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Parameters

Parameter	Description	Units
culture_ID	sample identification	text
location	site description; NP is North Pacific Gyre near Axial seamount; PS is Puget Sound main basin	text
lat	Latitude	decimal degrees
lon	Longitude; West is negative	decimal degrees
depth	Sampling depth	meters
month	month sampling took place	text
year	year sampling took place	number
NCBI_Accession	link to NCBI GenBank	link
Bayesian_Classification	statistical taxonomic assignments using method of Wang et al.(2007) ; p-values at each taxonomic level shown are greater than 0.7	text

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Deployments

TN268

Website	https://www.bco-dmo.org/deployment/626431
Platform	R/V Thomas G. Thompson
Start Date	2011-08-11
End Date	2011-09-01
Description	This was a two leg cruise. The National Science Foundation's Ocean Observatory Initiative-Regional Scale Nodes cruise (August 19 – September 1, 2011) from Seattle, WA to Hydrate Ridge and Axial Seamount. The cruise began August 11 when it left the port of Seattle. **Methods & Sampling** Culturing experiments were conducted with surface water collected from the Puget Sound main basin (47° 41.24' N, 122° 24.14' W) in November 2009 and from the DCM (45 m) in the North Pacific gyre near Axial seamount in August 2011.

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Project Information

Mixotrophic bacteria and the cryptic marine sulfur cycle: Mechanisms of carbon assimilation and sulfur oxidation in the Arctic96BD-19 GSO clade (Sulfur Oxidizers)

Website: http://morrislab.ocean.washington.edu/

Coverage: North Pacific Ocean

Description from NSF award abstract:
The ocean serves an immense reservoir of carbon, nitrogen, phosphorus, sulfur, and other elements required for all life. The active and diverse microbial populations that inhabit the oceans are responsible for mediating nutrient transformations that maintain the chemistry of seawater. A recent study identified a ubiquitous group of marine bacteria from the Arctic96BD-19 gamma-proteobacterial sulfur oxidizer (GSO) lineage that is closely related to known sulfur oxidizing species that fix inorganic carbon and oxidize sulfide in low-oxygen waters. The potential for GSOs to use reduced forms of sulfur in oxygenated waters suggests that they are a keystone species that link the marine carbon and sulfur cycles. The only known isolates from the Arctic96BD-19 lineage of GSOs are now in culture, allowing fundamental questions about their roles in carbon and sulfur cycling to be investigated. Preliminary data suggest that they use energy from the oxidation of sulfur to assimilate carbon. This project seek to address the overarching hypothesis that sulfur transformations provide the Arctic96BD- 19 lineage of GSOs with energy for organic and inorganic carbon cycling throughout the water column.

Three specific hypotheses will be tested.
1. Arctic96BD-19 cells assimilate either organic carbon or fixes inorganic carbon, depending on environmental conditions.
2. Arctic96BD-19 cells oxidize thiosulfate via formation of a tetrathionate intermediate, or using the branched thiosulfate oxidation pathway.
3. Arctic96BD-19 cells are ubiquitous sulfur oxidizers that assimilate organic and inorganic carbon through the Pacific Northwest.

A combination of laboratory growth studies of the investigator's pure cultures and comparative genomic analyses will be used. The genomic data will be used to determine whether the Arctic96BD-19 cultures possess the genetic potential to oxidize reduced sulfur to sulfate (based on possession of known core and ancillary sulfur oxidation genes), which potential oxidation pathways are used, and whether they can fix inorganic carbon. These data will help guide the physiology studies by determining the most likely forms of inorganic and organic compounds that can be utilized.

Marine bacteria are critical players in global nutrient cycles, but many of their individual and community functions in the ecosystem are not well understood. Future oceanographers will need to use cultivation-dependent and cultivation-independent methods to identify metabolic process that shape microbial communities and impact biogeochemical cycles. Student education, scientific advancement, and public awareness are all important components of this project.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1232840

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