# pmoA sequences amplified from DNA extracted from Whillans Subglacial Lake sediment in West Antarctica

**Website**: https://www.bco-dmo.org/dataset/823123
**Version**: 1
**Version Date**: 2020-09-02

**Project**
» Microbial carbon cycling beneath the West Antarctic Ice Sheet (SLW C Cycling)

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

| Contributors | Affiliation | Role |
|---|---|---|
| Michaud, Alexander | Montana State University | Principal Investigator |
| Priscu, John C. | Montana State University | Contact |
| York, Amber D. | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |

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## Coverage

**Spatial Extent**: **N**:-83.79 **E**:-131.77 **S**:-85.02 **W**:-155.33

## Dataset Description

These are pmoA sequences amplified from DNA extracted from Whillans Subglacial Lake sediment to understand the microorganisms responsible for methane oxidation. The pmoA sequences have been deposited in GenBank under accession numbers KX589304-KX589461 (see https://www.ncbi.nlm.nih.gov/popset/1231961266) and KX784213-KX84230 (see https://www.ncbi.nlm.nih.gov/popset/1231961582).

The MiMARKS forms can be found in the Microbial Antarctic Resource System (mARS) under resource http://mars.biodiversity.aq/resources/198. MiMARKS = Minimum information about a marker gene sequence.

### Methods & Sampling

Location:
Samples were collected from Subglacial Lake Whillans, at the lower portion of the Whillans Ice Stream, West Antarctica. Samples were collected through a 800m borehole created with a clean, hot water drill system. Coordinates: 85°1'12"S and 83°47'24"S Latitude; 155°19'48"W and 131°46'12"W Longitude

Methodology:

Planktonic microbial cells were collected on 0.2, 0.8, 3.0, and 10.0 micron filters and community structure was determined from 16S rRNA gene identities. Microbial community structure was also determined for samples from a shallow sediment core (0-40 cm).

For full methodology details refer to Christner et al., 2014. Further detailed methods for microbial community composition can be found in Achberger et al., 2017.

Design description:

A hot water drilling system was used between 23–27 January 2013 to melt through the ~801 m thick ice sheet, creating an access borehole (minimum diameter ~60 cm) for direct sampling and to conduct in situ measurements of the SLW water column and sediments. Microbial cells in the drilling water and on exposed surfaces of the hose, cables, and deployed equipment were reduced and killed through the use of four complementary technologies: (1) filtration, (2) ultraviolet irradiation, (3) pasteurization, and (4) disinfection with 3% w/v $H_2O_2$. The drilling water, derived from the overlying ice sheet, was continuously circulated through a water treatment system that removed micron and sub-micron sized particles (>0.2 μm), irradiated the drilling water with two germicidal wavelengths of ultraviolet radiation (185 nm ~40,000 μW $s^{-1}$ $cm^{-2}$ and 254 nm ~175,000 μW $s^{-1}$ $cm^{-2}$), and pasteurized the water at 90 °C to reduce the viability of persisting microbial contamination. Ports were plumbed along the system's flow path, allowing discrete water samples to be obtained before and after each stage. The drill hose and instrument cables were deployed at a rate no greater than 1 m $s^{-1}$ through a custom borehole collar that contained 12 amalgam pellet ultraviolet lamps, providing a cumulative germicidal ultraviolet dosage of at least 40,000 μW $s^{-1}$ $cm^{-2}$ (Arapahoe SciTech). All borehole sampling tools and instruments were spray-saturated with 3% w/v $H_2O_2$ and staged in sealed polyethylene bags until tool deployment. Single-use protective apparel (Tyvek) was worn by all personnel during borehole science operations. The efficacy of the clean access technology and procedures were tested thoroughly before use in the field.

Sampling description

Water samples were collected and brought to the surface in Niskin bottles. Particulate matter for DNA sequence analyses was collected on 10.0, 3.0, 0.8, and 0.2 micron filters by an in situ filtration unit. Sediment samples were collected and brought to the surface using a shallow sediment multicorer.

Analytical Methods:

Sample Processing

SLW sediment porewater samples were collected from multicore 3B (MC-3B) using a Rhizon porewater sampler (Rhizosphere). Rhizon porewater samplers were prepared by soaking in MilliQ (18.2 MΩ) water prior to installation through a pre-drilled hole in the sediment core liner. A 10 mL syringe was attached to the outlet and the plunger locked to maintain a vacuum. After 14 h of porewater extraction through the 0.2 μm filter on the end of the Rhizon, the porewater was dispensed into cleaned bottles/vials (Major ions: 10% HCl acid washed and 6 MilliQ rinses; Trace elements: 10% trace metal grade $HNO_3$ acid washed and 6 MilliQ rinses; Water stable isotopes: new, filled with no headspace). SLW water samples for major ion analysis were filtered through a 0.4 μm polycarbonate filter (Whatman) and TE/REE samples were filtered through a 0.2 μm PTFE membrane syringe filter. The filtrate, or raw water in the case of the water stable isotope samples, was dispensed into a bottle cleaned as described above. Major ion and TE samples were frozen at -20°C and shipped to Montana State University and University of Venice, respectively, for analysis. Stable isotope samples were stored at 4°C and were shipped to University of Washington-Seattle for analysis. Procedural blanks were handled and processed in parallel to SLW samples. Briefly, MilliQ water from McMurdo Station was taken into the field in a 10% HCl acid washed and 6-time MilliQ rinsed HDPE bottle and was run through the same sampling set up for SLW water samples. For the porewater procedural blanks, rhizons were inserted into the MilliQ bottle and ~5 mL of water was pulled through the Rhizon into the syringe and allowed to reside in the syringe for 14 h to replicate syringe residence time. Procedural blank water was then dispensed into the three sample containers for subsequent analysis as described above.

Functional Gene Analyses

DNA was extracted using a modular method to allow for optimization of the DNA extraction procedure, specific to the SLW sediments. DNA extraction yield from SLW sediments was greatest when sediments were pre-treated with 450 µmol g-1 deoxynucleotide triphosphate to prevent adsorption of lysed DNA to the abundant clay particles in SLW44. The particulate methane monooxygenase (pmoA) gene clone libraries were constructed by PCR amplification using A189F (5' GGNGACTGGGACTTCTGG 3') and m680R (5' CCGGMGCAACGTCYTTACC 3'). The PCR was set up using 0.13 µL of ExTaq at 5 units µL-1 (Takara), 2.5 µL of 10x ExTaq buffer (Takara), 2 µL dNTP mixture at 2.5mM per nucleotide (Takara), 2.5 µL of A189F and Mb661R primers (10 pmol µL-1), 2 µL molecular biology-grade bovine serum albumen (BSA; 1.6 mg ml-1 final concentration) (New England BioLabs Inc.), 4 µL of template DNA (0.01-0.09 ng DNA µL-1), and 11.37 µL of PCR-grade water for a final reaction volume of 25 µL. The PCR thermocycling conditions were 1 cycle of 98°C for 2 min; 40 cycles of 98°C for 15 s, 55°C for 1 min, and 72°C for 1 min; followed by a final 72°C for 7 min. PCR was conducted with DNA extraction blanks and no template blanks (PCR-grade water) as negative controls. Negative controls were not carried forward for cloning, as no PCR bands were detected. PCR products were run on a 1.5% agarose gel and the 491 basepair pmoA fragment was excised from the gel with sterile razor blade and DNA was purified using a Wizard SV gel clean-up system (Promega). Cleaned pmoA fragments were immediately ligated and cloned with a TA Cloning kit (Invitrogen). Positive clones were transferred to LB+ampicillin broth and grown overnight at 37°C, then sequenced (288 total sequenced clones) (Functional Bioscience). The pmoA DNA sequences were processed by removing the forward and reverse primer sequences and removing poor quality sequences (<20 phred score). Quality controlled pmoA sequences (176 total) were clustered into operational taxonomic units (OTUs) at the 97% similarity level and one representative sequence from each OTU51, along with representative pmoA sequences from type Ia and II methanotrophs, were aligned using ClustalW using the default alignment parameters within the program MEGA6. A phylogenetic tree was built using the neighbor-joining method with 1000 bootstrap replications52. The pmoA sequences have been deposited in GenBank under accession numbers KX589304-KX589461 and KX784213-KX84230.

We attempted to amplify mcrA gene sequences from SLW sediment DNA extracts using a primer set designed to amplify the diversity of mcrA-containing methanogens with a nested PCR amplification scheme. The primer pair used to detect the mcrA gene sequence were mcrIRD. The primer pair is capable of detecting a wide diversity of known and several novel mcrA gene clusters. The first reaction was set up using 0.13 µL of Takara ExTaq at 5 units µL-1, 2.5 µL 10x ExTaq buffer, 2 µL dNTP mixture at 2.5mM per nucleotide (Takara), 2.5 µL of forward and reverse primer (10 pmol µL-1), 2 µL of BSA (1.6 mg ml-1 final concentration), 9.38 µL PCR-grade water and 4 µL DNA extract (0.01-0.09 ng DNA µL-1) for a final reaction volume of 25 µL. This first reaction was run with an initial denaturation step at 98°C for 2 minutes followed by 40 cycles of 98°C for 15 s, 53°C for 1 min and 72°C for 1 min, and a final elongation at 72°C for 7 min. The second reaction was set up using 0.25 µL Takara ExTaq, 5 µL 10x ExTaq buffer, 4 µL dNTP mixture at 2.5mM per nucleotide (Takara), 5 µL of forward and reverse primers (10 pmol µL-1), 4 µL of BSA (1.6 mg ml-1 final concentration), 21.75 µL of PCR-grade water and 4 µL of product from the first reaction as template DNA. The second reaction was run with the same thermocycler program as the first reaction. PCR was conducted with DNA extraction blanks and no template blanks (PCR-grade water) as negative controls. Extraction blanks were conducted, processed and analyzed in parallel with the SLW sediment samples.

**Data Processing Description**

Paired end sequence reads were assembled and quality filtered using the Mothur phylogenetic analysis pipeline (v1.33.2). The sequences were aligned with the SILVA Incremental Aligner47 (SINA v1.2.11; database release 115). The aligned reads were checked for chimaeras using the Uchime algorithm, as implemented within Mothur, and chimaeric sequences were removed from the data. Sequences with >97% SSU rRNA gene sequence similarity were clustered into an OTU and representative sequences for each OTU were chosen for classification using the SILVA database.

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## Related Publications

Achberger, A. M., Christner, B. C., Michaud, A. B., Priscu, J. C., Skidmore, M. L., & Vick-Majors, T. J. (2016).

Microbial Community Structure of Subglacial Lake Whillans, West Antarctica. Frontiers in Microbiology, 7. doi:10.3389/fmicb.2016.01457
*Results*

Christner, B. C., Priscu, J. C., Achberger, A. M., Barbante, C., Carter, S. P., … Vick-Majors, T. J. (2014). A microbial ecosystem beneath the West Antarctic ice sheet. Nature, 512(7514), 310–313. doi:10.1038/nature13667
*Results*

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## Related Datasets

### IsSupplementedBy

Michaud, A., Priscu, J. C. (2020) **Geochemical data collected from Whillans Subglacial Lake sediment cores in West Antarctica.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). Version Date 2020-08-27 http://lod.bco-dmo.org/id/dataset/822288 [view at BCO-DMO]
*Relationship Description: Related geochemistry data from the same samples.*

### IsSupplementTo

Michaud, A., Priscu, J. C. (2020) **Geochemical data collected from Whillans Subglacial Lake sediment cores in West Antarctica.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). Version Date 2020-08-27 http://lod.bco-dmo.org/id/dataset/822288 [view at BCO-DMO]

### References

Michaud,A.B., Dore,J.E., Achberger,A.M., Christner,B.C., Mitchell,A.C., Skidmore,M.L., Vick-Majors,T.J. and Priscu,J.C. (2017). Uncultured bacterium particulate methane monooxygenase subunit A (pmoA) gene, partial cds. The National Center for Biotechnology Information PopSet: 1231961266. Available from https://www.ncbi.nlm.nih.gov/popset/1231961266

Michaud,A.B., Dore,J.E., Achberger,A.M., Christner,B.C., Mitchell,A.C., Skidmore,M.L., Vick-Majors,T.J. and Priscu,J.C. (2017). uncultured bacterium particulate methane monooxygenase subunit A (pmoA) gene, partial cds. The National Center for Biotechnology Information PopSet: 1231961582. Available from https://www.ncbi.nlm.nih.gov/popset/1231961582

Priscu J (2019). Geomicrobiology of Antarctic Subglacial Environments - Subglacial Lake Whillans. GBASE2020_part2. Version 1.1. SCAR - Microbial Antarctic Resource System. http://mars.biodiversity.aq/resources/198

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## Parameters

*Parameters for this dataset have not yet been identified*

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## Project Information

### Microbial carbon cycling beneath the West Antarctic Ice Sheet (SLW C Cycling)

**Coverage**: 800 m beneath the Whillans Ice Stream (84.24°S, 153.69°W)

Project description from C-DEBI (https://www.darkenergybiosphere.org/award/microbial-carbon-cycling-beneath-the-west-antarctic-ice-sheet/)

Subglacial lakes were discovered beneath the Antarctic Ice Sheet in the 1970's and, given the presence of liquid water and saturated sediments, it has been debated whether or not these deep, cold biosphere habitats harbor active microbial communities. Subglacial Lake Whillans (SLW) was cleanly sampled in January 2013 with the goal of establishing the habitability and presence of life beneath the Antarctic Ice Sheet. The aim of this graduate fellowship project was to further characterize the SLW carbon cycle, in particular, chemolithoautotrophic microbial processes through geochemical and microbiological methods. Geochemical analyses showed that sulfide oxidizing bacteria were active and contribute to mineral weathering in the surficial sediments of SLW. Long water residence times beneath the West Antarctic Ice Sheet (WAIS) create a mineral weathering regime in SLW that is distinctly different from subglacial habitats of mountain glaciers. Concentration and stable isotope measurements of methane confirm a reservoir of methane formed by methanogenic archaea beneath the WAIS. The modeling results show that this biological methane provides a source of energy to an active methane oxidizing population at the sediment-water interface. The methane also is modeled to be an important source of carbon for biomass synthesis in the methane oxidizing population, with rates of biomass incorporation similar to that of ammonia oxidizing archaea in the SLW water column. These results provide evidence that the sub ice sheet environment provides favorable conditions and substrates to support an active microbial ecosystem, thus expanding the extent of the biosphere to include the area beneath the WAIS and, possibly, the entire Antarctic Ice Sheet.

This project was funded by a C-DEBI graduate fellowship.

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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 |
| NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP) | OPP-1023233 |
| NSF Office of Polar Programs (formerly NSF PLR) (NSF OPP) | OPP-1115245 |


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