The manually curated genome of the SAG SCGC AD-561-N23; collected from R/V JOIDES Resolution JRES-318 from Wellington, New Zealand to Hobart, Australia from January to March 2010

Website: https://www.bco-dmo.org/dataset/654189
Data Type: Cruise Results
Version: 16 Aug 2016
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Project
» Functional potential of the uncultivated Candidate Phylum OP9 from deep Antarctic marine sediments using single cell genome techniques (Adelie Basin Atribacteria)

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

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Dataset Description

This is the manually curated genome of the SAG SCGC AD-561-N23. Sampling occurred in the Adelie Basin during the Integrated Ocean Drilling Program (IODP) Expedition 318, aboard R/V JOIDES Resolution, Site U1357.

Methods & Sampling

Samples for molecular biological analysis were collected shipboard from 10 cm sections of the intact core using sterile cut-end 5 mL syringes. Samples were immediately frozen at –80 degrees C and maintained at this temperature during transport and storage to the home laboratory. Sorting of individual cells from unfixed frozen sediment from 97.41 mbsf was attempted for single cell genomics using the Single Cell Genomics Center (SCGC) at the Bigelow Laboratory for Ocean Sciences. While the sediment was not preserved with recommended fixatives to prevent cell lysis during storage, and although the samples had gone through at least one round of freeze-thaw for bulk DNA extraction which may have introduced cell lysis, the high relative abundance of target microorganisms in the sample suggested this approach might still be successful in recovering intact cells. Approximately 0.5 g of frozen sediment was diluted in 1 mL of filter-sterilized seawater and vortexed for 30 s to liberate cells from the sediment matrix, modifying methods developed previously. Sediment was then separated from cells by gentle centrifugation at 2000 rpm for 30 s The cell suspension was treated with SYTO-9 DNA stain and sorted into two 384-well plates using SCGC's s tandard pipeline. Both sorted plates were subjected to physical lysis treatments (five freeze- thaw cycles), and the second plate also

experienced an alkaline lysis treatment. DNA amplification by multiple displacement amplification.

Data Processing Description

The initial assembly of Atribacteria bacterium SCGC AD- 561-N23 is publically available within the IMG system (taxon ID 2588254308) and the sequence for the 16S rRNA gene is available within the IMG system and Supplementary Materials. A detailed assembly procedure (QC.finalReport.pdf) can be downloaded from: http://genome.jgi.doe.gov/CandivSCAD561N23/CandivSCAD561N23.download.html.

Briefly, single-cell amplified genomic (SAG) DNA was sequenced, assembled and annotated at the United States Department of Energy's Joint Genome Institute (JGI) following their standard pipeline for Illumina HiSeq 2000 platform sequencing. Illumina reads were screened using JGI's in-house DUK filtering program (Mingkun et al., unpublished). Trimmed reads were assembled using SPAdes (version 3.0.0) with the following parameters (–t 8 –m 40 – –sc – –careful – –12; Bankevich et al., 2012). Once released to Integrated Microbial Genomes (IMG) system, manual screening and removal of potential contaminate sequences according to JGI's single cell data decontamination protocol (Clingenpeel, 2015). Scaffolds with GC contents that varied from the genome average more than 10% and clustered as a distinct group according to a kmer analysis (IMG, fragment window 5000 bp, fragment step 500 bp, oligomer size 5, minimum variation 10) were identified as potential contaminates and were removed from the de novo assembly (with the exception of scaffolds that contained ribosomal DNA). This screened genome was submitted to the IMG database as GOLD project Gp0087948, titled "Candidate division JS 1 bacterium SCGC AD-560-N23 (manually screened)". Gene annotations were performed using both IMG and the Rapid Annotation using Subsystem Technology (RAST) platforms (Aziz et al., 2008; Overbeek et al., 2013 ;Markowitz et al., 2014). Discrepancies between annotations were investigated by comparing coding sequences of genes against GenBank non-redundant protein sequence and Swiss- Prot Databases by BLASTP (Altschul et al., 1990). Genome
completeness was estimated by comparing the annotated genome sequence against a list of conserved single copy bacterial genes.

This screened genome was submitted to the IMG database as GOLD project Gp0087948 with an IMG taxon ID of 2626541500, and to MG-RAST under accession numbers 4624791.3–4634830.3.

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Parameters

Parameters for this dataset have not yet been identified

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Deployments

JRES-318

Website	https://www.bco-dmo.org/deployment/654178
Platform	R/V JOIDES Resolution
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/318PR.PDF
Start Date	2010-01-03
End Date	2010-03-08
Description	More information about this cruise is available from IODP: https://iodp.tamu.edu/scienceops/expeditions/wilkes_land.html

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Project Information

Functional potential of the uncultivated Candidate Phylum OP9 from deep Antarctic marine sediments using single cell genome techniques (Adelie Basin Atribacteria)

Coverage: Antarctic Coast

Bacteria belonging to the newly classified candidate phylum “Atribacteria” (formerly referred to as “OP9” and “JS1”) are common in anoxic methane-rich sediments. However, the metabolic functions and biogeochemical role of these microorganisms in the subsurface remains unrealized due to the lack of pure culture representatives. In this study of deep sediment from Antarctica’s Adélie Basin, collected during Expedition 318 of the Integrated Ocean Drilling Program (IODP), Atribacteria-related sequences of the 16S rRNA gene were abundant (up to 51% of the sequences) and steadily increased in relative abundance with depth throughout the methane-rich zones. To better understand the metabolic potential of Atribacteria within this environment, and to compare with phylogenetically distinct Atribacteria from non-deep-sea environments, individual cells were sorted for single cell genomics from sediment collected from 97.41 m below the seafloor from IODP Hole U1357C. As observed for non-marine Atribacteria, a partial single cell genome suggests a heterotrophic metabolism, with Atribacteria potentially producing fermentation products such as acetate, ethanol, and CO2. These products may in turn support methanogens within the sediment microbial community and explain the frequent occurrence of Atribacteria in anoxic methane-rich sediments. This first report of a single cell genome from deep sediment broadens the known diversity within the Atribacteria phylum and highlights the potential role of Atribacteria in carbon cycling in deep sediment.

This project was funded by a C-DEBI Postdoctoral 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

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