Coastal eastern subtropical pacific

Website: https://www.bco-dmo.org/dataset/745645
Version: 1
Version Date: 2018-09-06

Project
» Gene content, gene expression, and physiology in mesopelagic ammonia-oxidizing archaea (AmoA Archaea)

Contributors	Affiliation	Role
Dupont, Christopher	J. Craig Venter Institute (JCVI)	Principal Investigator, Contact
Santoro, Alyson E.	University of California-Santa Barbara (UCSB)	Co-Principal Investigator
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Nitrosopelagicus braves CN25 and U25 were grown in nitrogen replete and deplete conditions, with subsequent transcriptome sequencing. The genome is available at The National Center for Biotechnology Information (NCBI) under accession number LXWN00000000.

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Coverage

Spatial Extent: **Lat**:15 **Lon**:-173
Temporal Extent: 2011-10-23

Dataset Description

These data were published in Carini et al., 2018.

Methods & Sampling

Organism sources and cultivation conditions:
'Candidatus Nitrosopelagicus brevis' str. CN25 (Santoro et al., 2015), was propagated in ONP medium (Santoro and Casciotti, 2011), consisting of aged natural seawater (collected from 10 m depth at 15°S, 173°W on 23 October 2011; 0.2 µm pore size filtered at sea) amended with a chemolithoautotrophic nitrogen source (NH_4Cl or urea, described in 'Experimental design'), ampicillin (10.8 µM), streptomycin (68.6 µM), potassium phosphate (29.4 µM), and a chelated trace metal mix consisting of disodium ethylenediaminetetraacetic acid (14 µM), $FeCl_2$ (7.25 µM), $ZnCl_2$ (0.5 µM), $MnCl_2$ (0.5 µM), H_3BO_3 (1 µM), $CoCl_2$-$6H_2O$ (0.8 µM), $CuCl_2$-$2H_2O$ (0.1 µM), $NiCl_2$-H_2O (0.1 µM), Na_2MoO_4-$2H_2O$ (0.15 µM). 'Ca. N. brevis' str. U25 was enriched from the original CN25 enrichment culture (Santoro and Casciotti, 2011) using sequential transfers of the initial enrichment into ONP medium amended with 50-100 µM urea, instead of NH_4Cl, over a period of ~48 months. All enrichments were propagated in 250 mL polycarbonate flasks at 22°C in the dark and monitored for NO_2^- production using the Griess reagent colorimetric method (Strickland and Parsons, 1972). Cell counts were

obtained with a Millipore Guava EasyCyte 5HT flow cytometer as described previously (Tripp, 2008).

Cell harvesting for and genome sequencing of ‘Ca. N. brevis’ str. U25: A Ca. N. brevis U25 enrichment culture that was grown exclusively with urea as the sole chemolithoautotrophic growth substrate for >50 generations, was harvested by filtration on to 25 mm diameter, 0.22 µm pore-size Supor-200 filters and frozen at -80°C. DNA was extracted using a DNeasy blood & Tissue DNA extraction kit (Qiagen, Valencia, CA, USA), following the manufacturer’s instructions. The DNA was treated with RNAse and examined using a Bioanlayzer 2100 (Agilent) with 500 ng serving as the input for library construction (NEBNext paired-end DNA Library Prep kit, New England Biolabs).

Instruments:
The sample was sequenced on an Illumina MiSEQ (v2 chemistry, paired 250 bp reads). Reads were quality trimmed and served as the inputs to assembly with metaSPAdes (v 0.5, 70mer) (Nurk et al., 2017). The K-mer usage of and phylogenetic annotation of the assembled contigs were then used to visually identify a putative thaumarchaeal bin (Supplementary Figure 1a) (Laczny et al., 2015). The 3 contig genome was annotated using the JGI IMG pipeline and the PGAP pipeline at NCBI.

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Related Publications

Carini, P., Dupont, C. L., & Santoro, A. E. (2018). Patterns of thaumarchaeal gene expression in culture and diverse marine environments. Environmental Microbiology. https://doi.org/10.1111/1462-2920.14107
Results

,
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Illumina MiSEQ
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina MiSEQ (v2 chemistry, paired 250 bp reads)
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Millipore Guava EasyCyte 5HT flow cytometer
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

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Project Information

Gene content, gene expression, and physiology in mesopelagic ammonia-oxidizing archaea (AmoA Archaea)

Coverage: Epipelagic and mesopelagic, Equatorial Pacific

NSF award abstract:

Intellectual Merit. How organisms respond to their physical and chemical and environment is a central question in marine ecology. For microbes living in the mesopelagic - the ocean's "twilight zone" - an efficient response is particularly important to capitalize on the intermittent delivery of organic and inorganic compounds sinking from the surface ocean. These organisms must have a suite of metabolic and regulatory strategies used to cope with environmental variability, but these strategies are largely unknown. Understanding when and why metabolic genes are expressed is critical to our understanding of nutrient remineralization in the ocean. Marine group 1 (MG1) archaea are ubiquitous, abundant microbes in the meso- and bathypelagic and promising model organisms for investigating these questions. MG1 archaea are chemolithoautotrophs that oxidize ammonia for energy and fix carbon for biomass, and as such, play a central role in the ocean's coupled carbon and nitrogen cycles. Though MG1 have historically eluded cultivation, recent efforts have been successful at bringing representative MG1 archaea from the open ocean into culture and demonstrating their importance in the production of the greenhouse gas nitrous oxide. This project takes advantage of unique MG1 cultures and the recently sequenced draft genome of one of the organisms - strain CN25 - to investigate the physiological and transcriptional responses of MG1 archaea to variations in their chemical environment, specifically:

1. Comparative transcriptomics of CN25 cells grown under a range of energy availability and nitrosative stress will identify select genes that can be used to diagnose the physiological state of natural populations

2. Improvements in the genomic and transcriptomic knowledge of MG1 archaea will facilitate a thorough reinterpretation of existing metagenomic and metatranscriptomic datasets, as well as provide a better contextual understanding in future studies

The investigators will conduct comparative transcriptomics of CN25 cells harvested in mid-exponential growth and stationary phase versus starved cells. Transcriptomes of cells grown at high nitrate concentrations and low pO2 with those grown in standard conditions will be characterized. A strand-specific, high-density RNAseq approach will be used to examine the expression of putative ORFs, polycistronic operons, and small RNAs, which, in addition to gene expression profiling, has the ancillary benefit of improving genome annotation. Finally, the investigators will sequence the genomes of two additional MG1 strains isolated from the open ocean, as well as single cells from environmental surveys, and leverage the combination with the CN25 genome to reanalyze available metagenomic and metatranscriptomic datasets. The results will define the transcriptional response of a model mesopelagic microbe to a range of chemical environments, and show how the physicochemical environment induces changes in gene expression and gene content that result in greenhouse gas production. This work will rapidly generate new knowledge of how some of the most ubiquitous, yet

heretofore elusive, microorganisms respond to geochemical variability and shape our evolving understanding of the marine nitrogen cycle.

Broader Impacts. The scientific and societal impact of the project will be to elucidate the mechanisms of greenhouse gas production in a model marine organism that is of broad interest to biological and chemical oceanographers. Transcriptome sequencing will improve the assembly of the CN25 genome, the first genome of an MG1 archaeon from the open ocean. Both the genome and transcriptomes will be important references for researchers using metagenomics, metatranscriptomics, and metaproteomics in the ocean, as these techniques are reliant on a knowledgebase composed of both DNA sequence and physiology. Thus, the results add value to both existing and future studies. The proposed research will advance education, teaching, and training for the next generation of marine scientists by providing support for two early-career investigators, one postdoctoral researcher, and a secondary school teacher.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1259994
NSF Division of Ocean Sciences (NSF OCE)	OCE-1260006

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