Nitrosopelagicus brevis CN25 and U25 grown in nitrogen replete and deplete conditions, with subsequent transcriptome sequencing and identification.

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Project

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

Contributors	Affiliation	Role
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Abstract

Nitrosopelagicus brevis CN25 and U25 were grown in nitrogen replete and deplete conditions, with subsequent transcriptome sequencing.

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Coverage

Spatial Extent: Lat:38.57506 Lon:-76.070137

Dataset Description

Nitrosopelagicus brevis CN25 and U25 were grown in nitrogen replete and deplete conditions, with subsequent transcriptome sequencing.

Methods & Sampling

Thaumarchaea transcriptomes sequences.

Data Processing Description

Libraries were sequenced in one 300 cycle NextSEQ run. Raw Illumina reads in fastq format are interleaved to

match paired ends. Sequencing primers and barcode indexes were identified by BLAST against the NCBI vector database and trimmed along with regions with Q scores < 30. Reads mapping to ribosomal RNAs were identified and removed using ribopicker.

Reads were mapped (clc_ref_assemble_long -s 0.9, CLC genomics) to the Ca. N. brevis strains CN25 or U25 genome sequences. Raw read counts per open reading frame (ORF) were compiled.

BCO-DMO Data Processing Notes:

- Included links to NCBI via accession numbers
- Reformatted column names to comply with BCO-DMO standards

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Data Files

File
accessions.csv(Comma Separated Values (.csv), 2.84 KB) MD5:6e045bed251d5f73a98fd794b7d192ad
Primary data file for dataset ID 739636

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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/<u>10.1111/1462-2920.14107</u> *Results*

Methods

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Parameters

Parameter	Description	Units
Accession	Accession number and NCBI link	unitless
Sample_Name	Sample name	unitless
Organism	Species of organism	unitless
Tax_ID	Taxonomic identification	unitless
Strain	Strain identification	unitless

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Instruments

Dataset- specific Instrument Name	Illumina NextSEQ 500
Generic Instrument Name	Automated DNA Sequencer
Dataset- specific Description	Used to identify samples
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

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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)	<u>OCE-1259994</u>
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