Laboratory results containing transcripts of four cultivated marine bacteria from 2014-2015 (EAGER Respiration project)

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Project
» Coordination of respiratory gene transcription and respiration in cultivated marine bacteria (EAGER Respiration)

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Dataset Description

The RNA-seq data have been deposited with NCBI under GenBank Accession Number PRJNA276979.

Bacteria respond to stimuli in the environment using transcriptional control, but this may not be the case for most marine bacteria having small, streamlined genomes. Candidatus Pelagibacter ubique, a cultivated representative of the SAR11 clade, which is the most abundant clade in the oceans 4, has a small, streamlined genome and possesses an unusually small number of transcriptional regulators. This observation leads to the hypothesis that transcriptional control is low in Pelagibacter and limits its response to environmental conditions. However, the extent of transcriptional control in Pelagibacter is unknown. Overall design: Bacteria were grown in batch culture and sampled twice during the initial, rapid phase of exponential growth and twice during the phase of slower growth that followed. Transcripts deposited here were assessed using RNA-seq.

Methods & Sampling

Sampling and Analytical Methodology

Bacterial strains. Pelagibacter ubique strain HTCC1062 and Gammaproteobacteria SAR92 strain HTCC2207 were obtained from S. Giovannoni (Oregon State University). Polaribacter strain MED152 was obtained from J. Pinhassi (Linnaeus University, Sweden). Ruegeria pomeroyi strain DSS-3 was obtained from Mary Ann Moran (University of Georgia).

Growth media and conditions. Pelagibacter and SAR92 were grown in a defined medium containing artificial seawater salts AMS1 (Carini et al. 2013) with additions of pyruvate, glycine, methionine and other organic substrates plus vitamins as described by (Steindler et al. 2011). Ruegeria was grown using YTSS medium that includes 0.4 g L-1 yeast extract and 0.25 g L-1 tryptone added to artificial seawater salts (Sigma-Aldrich). MED152 was grown in a medium with 5 g L-1 of peptone and 1 g L-1 of yeast extract added to the AMS1 salt solution. Cultures were maintained at 19°C in the dark and were aerated by bubbling with 0.2 µm filtered air or by rotary shaking.

Growth rates. Bacterial abundance was monitored by flow cytometry using a BD FACSCalibur of samples

stained with SYBR Green I (Invitrogen) at a concentration of 1:2,000 of the manufactures supplied concentration for 30 min. Growth rates were calculated from the rate of change of bacterial abundance over time.

Nucleic acid extraction. Bacterial biomass was collected by vacuum filtration using 0.2 µm-pore-size Durapore (Millipore) filters. The filters were stored at -80°C in RLT buffer until DNA and RNA were extracted using the AllPrep DNA/RNA (Qiagen) kit following the manufacturer's instructions.

RNA sequencing abundance standard. Internal standard RNA molecules were used to obtain absolute quantification of transcripts based on the number of standard molecules added at the beginning of sample processing and those recovered in the sequence library (Moran et al. 2013, Satinsky et al. 2013). RNA standards were prepared using in vitro transcription (RiboMax Large Scale RNA Production Systems, Promega) from plasmid templates pTXB1 (New England BioLabs) and pFN18K (Promega), yielding single stranded RNA transcripts of 917 nt and 970 nt, respectively. The RNA standards were added immediately before nucleic acid extraction at a concentration of 0.5% by mass of the total RNA yield in the sample (Moran et al. 2013, Satinsky et al. 2013).

Data Processing Description

Sequencing and analysis. RNA libraries were prepared for sequencing using Ribo-Zero rRNA Removal Kit (Bacteria) and TruSeq RNA Sample Prep Kit following Illumina protocols. Sequences were run on an Illumina HiSeq 2500 instrument, generating paired-end reads using the 2x150 cycle protocol.

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Parameters

Parameter	Description	Units
Genbank Accession Number	GenBank Accession Number corresponding to the RNA-seq data deposited with NCBI.	dimensionless

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Instruments

Dataset-specific Instrument Name	Illumina HiSeq 2500
Generic Instrument Name	Automated DNA Sequencer
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

Coordination of respiratory gene transcription and respiration in cultivated marine bacteria (EAGER Respiration)

Coverage: Laboratory in Lewes, DE: 38.785289 N, 75.160167 W

Heterotrophic bacteria account for about half of the respiration in the oceans and they are the main consumers of dissolved organic materials, converting approximately 80% of assimilated organic carbon to carbon dioxide. Detailed examinations of the structure of microbial communities reveal that a highly diverse community of microorganisms must be responsible for the conversion of organic materials in the oceans. Recent studies suggest that most of growth-related activity is associated with a rather small number of abundant bacteria in the oceans. It is unclear, however, if the same observation applies to respiration, the dominant fate of organic carbon in the oceans. We currently lack tools for assessing respiration by specific microbes and for linking specific taxa with their contribution to carbon conversion and to carbon cycling.

The aim of this project is to develop a tool for understanding the contribution of bacterial groups to bacterial respiration. The proposed work will determine the relationship between transcription levels of oxidative phosphorylation genes and respiration rates in cultivated marine bacteria. The culture-based work is a necessary prerequisite to develop a metatranscriptomic tool for addressing ecological questions about the role of bacterial diversity in ocean carbon cycling. The project will include chemostat and batch culture studies with pure cultures and an incubation experiment with a naturally community of estuarine bacteria. Laboratory experiments will examine important heterotrophic bacteria in the oceans such as Pelagibacter ubique of the SAR11 clade and Ruegeria pomeroyi of the Roseobacter clade. Existing genome sequences will be analyzed to address the phylogenetic resolving power of respiration genes. The overarching goal of this proposal is to determine the relationship between abundances of respiration gene transcripts and respiration rates for bacterial taxa defined at different phylogenetic distances.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1343773

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