Transcriptomics of phytoplankton cultures grown on various phosphorus sources in a laboratory experiment

Website: https://www.bco-dmo.org/dataset/949777
Data Type: experimental
Version: 1
Version Date: 2025-02-17

Project
» Phosphonate Utilization by Eukaryotic Phytoplankton: Who, How, and Where? (Euk Phn Utilization)

Contributors	Affiliation	Role
Lomas, Michael W.	Bigelow Laboratory for Ocean Sciences	Principal Investigator
Whitney, LeAnn P.	Maine Maritime Academy (MMA)	Co-Principal Investigator
Sterling, Hannah	Bigelow Laboratory for Ocean Sciences	Technician
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
A laboratory experiment was carried out to characterize the growth and physiological response of three species of eukaryotic phytoplankton grown with inorganic phosphate (+P), without phosphate (-P), and with methylphosphonate (+MPN) and aminoethylphosphonate (+AEPN) as the sole sources of phosphorus (P). Data reported in this dataset are the transcriptomic reads, in biological triplicate, of these culture growth substrate combinations.

Table of Contents

Coverage

Temporal Extent: 2022-05 - 2024-05

Methods & Sampling

Three species of marine phytoplankton – *Micromonas pusilla*, *Emiliania huxleyi*, and *Isochrysis galbana* - were grown under four phosphorus (P) conditions. These include phosphate (Pi) replete and deplete conditions and the phosphonate conditions where cultures received either methylphosphonate (MPN) or 2-aminoethylphosphonate (2-AEPN) as the sole source of phosphorus at replete levels.

Axenic cultures of the pico-prasinophyte *Micromonas pusilla* (CCMP1545), the coccolithophore *Emiliania huxleyi* (CCMP2090), and the pico-prymnesiophyte *Isochrysis galbana* (CCMP1323) were obtained from the National Center for Marine Algae and Microbiota (Bigelow Laboratory for Ocean Sciences, East Boothbay, Maine). The cultures remained axenic throughout the experiments as determined by SYTO-staining and flow cytometric counting on a BD FACSJazz cell sorter; all cultures were free of bacteria during these experiments. Phytoplankton were grown in artificial sea water amended with L1 media silica. The P source was added

separately to achieve the desired growth conditions; Pi-replete media contained 36 µM PO43-, the Pi-deficient condition received 0.1 µM PO43-, and the phosphonate treatments received either 36 µM MPN or 2-AEPN. The Pi-deficient treatment (0.1 µM) represents a control for the low level of contaminating Pi measured in the phosphonate media; thus, an increase in growth in the MPN and 2-AEPN conditions above that measured in the Pi-deficient condition is due to phosphonate utilization. The potential for abiotic breakdown of phosphonate to Pi was previously investigated in media-only tubes exposed to the experimental temperature and light conditions for 10 days. Pi levels did not change throughout the experimental period (MPN average Pi = 0.11 µM ± 0.02; 2-AEPN average Pi = 0.10 µM ± 0.02), strongly supporting the notion of active enzymatic breakdown of phosphonates for growth. Cultures were acclimated to the four growth conditions described above as they had been maintained in each P treatment for a minimum of two transfers (20 days). Cultures were grown at 20°C in a 14-hour light/10-hour dark cycle at ~100 µE m-2 s-1 with a starting concentration of ~1x104 cells per mL in 25 mL culture volumes. Phytoplankton growth was monitored by fluorescence measurements using a Turner TD-700 fluorometer and cell counts analyzed by flow cytometry. Triplicate cultures were setup for each treatment and were harvested in the late exponential phase of growth for transcriptomic analysis.

Data Processing Description

I. galbana RNA-seq data was trimmed of adapters using BBDuk v38.84 and quality controlled using FastQC v0.11.9. The transcriptome was assembled using Trinity v2.14.0 and quality controlled using TrinityStats.pl, BUSCO v5.4.3, Bowtie 2 v2.4.4. The transcriptome was filtered using Trinity and TransDecoder v5.5.0. The count matrix was generated using Bowtie2 and RSEM and differential expression determined using DESeq2 v1.38.1. Transcripts were functionally annotated using eggNOG-mapper v2.1.9, InterProScan v5.60-92.0, PANNZER2, KEGG, Blast2GO v6.0.3, and Diamond v2.0.14.

E. huxleyi and *M. pusilla* transcriptomes were trimmed of adapters using BBDuk 38.84 and quality controlled using FastQC v0.11.9, as above. Unlike *I. galbana*, *E. huxleyi* and *M. pusilla* have existing genomes; the transcripts were mapped to the genomes and count matrices were generating using STAR v2.7.10b. The transcripts were functionally annotated using InterProScan v5.60-92.0, Blast2GO v6.0.3, and Diamond v2.0.14. DESeq2 v1.38.1 was used analyze transcripts counts for differential expression.

BCO-DMO Processing Description

- Imported original file "Phn_Transcriptomics metadata_2024 update.xlsx" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved the final file as "949777_v1_phyto_transcriptomics.csv".

[table of contents | back to top]

Data Files

File
949777_v1_phyto_transcriptomics.csv (Comma Separated Values (.csv), 4.27 KB) MD5:1e259714d61f639bdf090e2585f498e9 Primary data file for dataset ID 949777, version 1

[table of contents | back to top]

Related Publications

Buchfink, B., Xie, C., & Huson, D. H. (2014). Fast and sensitive protein alignment using DIAMOND. Nature Methods, 12(1), 59–60. https://doi.org/10.1038/nmeth.3176
Methods

Cantalapiedra, C. P., Hernández-Plaza, A., Letunic, I., Bork, P., & Huerta-Cepas, J. (2021). eggNOG-mapper v2: Functional Annotation, Orthology Assignments, and Domain Prediction at the Metagenomic Scale. Molecular

Biology and Evolution, 38(12), 5825–5829. https://doi.org/10.1093/molbev/msab293
Methods

Conesa, A., Götz, S., García-Gómez, J. M., Terol, J., Talón, M., & Robles, M. (2005). Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21(18), 3674–3676. https://doi.org/10.1093/bioinformatics/bti610
Methods

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2012). STAR: ultrafast universal RNA-seq aligner. Bioinformatics, 29(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635
Methods

Emms, D. M., & Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biology, 20(1). https://doi.org/10.1186/s13059-019-1832-y
Methods

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., … Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nature Biotechnology, 29(7), 644–652. doi:10.1038/nbt.1883
Methods

Guillard, R. R. L., & Hargraves, P. E. (1993). Stichochrysis immobilis is a diatom, not a chrysophyte. Phycologia, 32(3), 234–236. doi:10.2216/i0031-8884-32-3-234.1
Methods

Jones, P., Binns, D., Chang, H.-Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A.F., Sangrador-Vegas, A., Scheremetijew, M., Yong, S-Y., Lopez, R., and Hunter, S. (2014). InterProScan 5: genome-scale protein function classification. Bioinformatics, 30(9), 1236–1240. doi:10.1093/bioinformatics/btu031
Methods

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology, 15(12). doi:10.1186/s13059-014-0550-8
Methods

Manni, M., Berkeley, M. R., Seppey, M., Simão, F. A., & Zdobnov, E. M. (2021). BUSCO Update: Novel and Streamlined Workflows along with Broader and Deeper Phylogenetic Coverage for Scoring of Eukaryotic, Prokaryotic, and Viral Genomes. Molecular Biology and Evolution, 38(10), 4647–4654. https://doi.org/10.1093/molbev/msab199
Methods

Moriya, Y., Itoh, M., Okuda, S., Yoshizawa, A. C., & Kanehisa, M. (2007). KAAS: an automatic genome annotation and pathway reconstruction server. Nucleic Acids Research, 35(Web Server), W182–W185. https://doi.org/10.1093/nar/gkm321
Methods

Soneson, C., Love, M. I., & Robinson, M. D. (2015). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Research, 4, 1521. https://doi.org/10.12688/f1000research.7563.1
Methods

Törönen, P., Medlar, A., & Holm, L. (2018). PANNZER2: a rapid functional annotation web server. Nucleic Acids Research, 46(W1), W84–W88. https://doi.org/10.1093/nar/gky350
Methods

[table of contents | back to top]

Related Datasets

IsRelatedTo

Bigelow Laboratory for Ocean Sciences. Phosphonate utilization by eukaryotic phytoplankton. 2024/10. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1172648. NCBI:BioProject: PRJNA1172648.

[table of contents | back to top]

Parameters

Parameter	Description	Units
BioProject	BioProject ID in NCBI (https://www.ncbi.nlm.nih.gov/)	unitless
Biosample	BioSample accession number	unitless
Treatment	Source of phosphorus in the growth media (+P: phosphate replete; -P: phosphate deplete; +MPN: methylphosphonate replete; +AEPN: aminoethylphosphonate	unitless
Replicate	Biological replicate sequenced	unitless
Organism	Taxonomic name of phytoplankton strain	unitless
Tax_ID	Genus species identification of strain	unitless
Strain	National Center for Marine Algae and Microbiota numerical designation (CCMPxxxx)	unitless
URL	URL of the BioSample	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	BD FACSJazz cell sorter
Generic Instrument Name	Automated Cell Counter
Generic Instrument Description	An instrument that determines the numbers, types or viability of cells present in a sample.

Dataset-specific Instrument Name	Illumina NovaSeq 6000
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	SAMN44278360 - SAMN44278371 (I. galbana) RNA-seq data were generated using the Illumina NovaSeq 6000 instrument at the University of New Hampshire. SAMN44278372 - SAMN44278383 (E. huxleyi) RNA-seq data were generated using the Illumina NoveSeq Plus instrument at the University of Chicago. SAMN44278384 - SAMN44278395 (M. pusilla) RNA-seq data were generated using the Illumina NoveSeq Plus instrument at the University of Chicago.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	flow cytometry
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)

Dataset-specific Instrument Name	Turner TD-700 fluorometer
Generic Instrument Name	Turner Designs 700 Laboratory Fluorometer
Generic Instrument Description	The TD-700 Laboratory Fluorometer is a benchtop fluorometer designed to detect fluorescence over the UV to red range. The instrument can measure concentrations of a variety of compounds, including chlorophyll-a and fluorescent dyes, and is thus suitable for a range of applications, including chlorophyll, water quality monitoring and fluorescent tracer studies. Data can be output as concentrations or raw fluorescence measurements.

[table of contents | back to top]

Project Information

Phosphonate Utilization by Eukaryotic Phytoplankton: Who, How, and Where? (Euk Phn Utilization)

Coverage: Laboratory culture studies

NSF Award Abstract:
Phosphorus (P) is an essential nutrient for all living cells. It is a central component of genetic material and cellular membranes and is integral to energy production and regulating enzyme activity. In the marine

environment, P occurs as inorganic (Pi) and dissolved organic (DOP) forms; the availability and concentration of these different forms of P is an important control on marine phytoplankton growth. Marine phytoplankton are single-celled photosynthetic organisms and can be both prokaryotic bacteria and eukaryotic plants. While Pi is the preferred form of P for marine phytoplankton, in large regions of the oceans it is at such low levels that it restricts phytoplankton growth. In these regions, DOP is the most important P source. The composition of the DOP pool can generally be divided into two major groups: P esters and phosphonates. All marine phytoplankton are capable of using P esters to support growth; in contrast, phosphonates have only been shown to be an important source of P in the nutrition of bacteria to date. This project will determine the ability of marine eukaryotic phytoplankton to use phosphonates as a source of P for growth. Genomic analyses will determine the metabolic response of eukaryotic phytoplankton species to growth on phosphonates as well as the relevance of phosphonate use by natural populations. It is critical to understand the metabolic capabilities of phytoplankton which control marine nutrient cycling. In addition, the project is of great value in understanding the potential impacts of a changing ocean on phytoplankton growth. The project supports reseach opportunities for undergraduates from a local community college as well as hands-on enrichment programs for an afterschool program that serves a diverse student population.

Comprising up to 10% of the marine DOP pool, phosphonates have been shown to be a dynamic P pool both being assimilated and produced by marine photosynthetic bacteria. The ability of eukaryotic phytoplankton to supplement their growth with phosphonates remains vastly unexplored. Several eukaryotic phytoplankton species have been shown to use glyphosate, a chemically synthesized herbicidal phosphonate, as a P source; it remains unknown if open ocean eukaryotic phytoplankton can utilize phosphonates found naturally in the marine environment. Preliminary experiments suggest at least some eukaryotic phytoplankton are able to directly utilize extracellular phosphonates. This project characterizes the pervasiveness of phosphonate utilization within eukaryotic phytoplankton lineages and identifies the cellular underpinnings that support the acquisition of and growth on naturally occuring phosphonates. The project uses whole-cell transcriptomics and functional gene complementation assays, in addition to phylogenetic analyses, to understand the bioavailability of phosphonates and relevance of phosphonate utilization by natural eukaryotic phytoplankton populations. It is critical to understand the metabolic capabilities of phytoplankton which control marine biogeochemical cycles. This is especially important given the prediction that future oceans may become more stratified which could increase the importance of DOP, including phosphonates, in supporting phytoplankton growth.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1756271

[table of contents | back to top]