Synechococcus DOP Hydrolysis Experiment - cell counts and IVF

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Project
» Collaborative Research: Assessing the role of compound-specific phosphorus hydrolase transformations in the marine phosphorus cycle (P-hydrolase)

Contributors	Affiliation	Role
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Abstract
Marine cyanobacterium Synechococcus (WH8102 and WH5701) laboratory culture in vivo fluorescence and flow cytometry cell counts following growth in high and low phosphate media, as part of the DOP hydrolysis experiments in a study of "Dissolved organic phosphorus bond-class utilization by Synechococcus" (Waggoner et al. submitted). Study Abstract: Dissolved organic phosphorus (DOP) contains compounds with phosphoester (P-O-C), phosphoanhydride (P-O-P), and phosphorus-carbon (P-C) bonds. Despite DOP's importance as a nutritional source for marine microorganisms, the bioavailability of each bond-class to the widespread cyanobacterium Synechococcus remains largely unknown. This study evaluates bond-class specific DOP utilization by cultures of an open ocean and a coastal ocean Synechococcus strain. Both strains exhibited comparable growth rates when provided phosphate, short-chain and long-chain polyphosphate (P-O-P), adenosine 5'-triphosphate (P-O-C and P-O-P), and glucose-6-phosphate (P-O-C) as the phosphorus source. However, growth rates on phosphomonoester adenosine 5'-monophosphate (P-O-C) and phosphodiester bis(4-methylumbelliferyl) phosphate (C-O-P-O-C) varied between strains, and neither strain grew on selected phosphonates. Consistent with the growth measurements, both strains preferentially hydrolyzed 3-polyphosphate, followed by adenosine 5'-triphosphate, and then adenosine 5'-monophosphate. The strains' exoproteome contained phosphorus hydrolases, which combined with enhanced cell-free hydrolysis of 3-polyphosphate and adenosine 5'-triphosphate under phosphate deficiency, suggests active mineralization of short-chain polyphosphate by Synechococcus' exoproteins. Synechococcus alkaline phosphatases presented broad substrate specificities, including activity towards short-chain polyphosphate, with varying affinities between the two strains. Collectively, these findings underscore the potentially significant role of compounds with phosphoanhydride bonds in Synechococcus phosphorus nutrition, thereby expanding our understanding of microbially-mediated DOP cycling in marine ecosystems.

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Coverage

Location: Laboratory experiments at the University of Arizona, Tucson, Arizona, US
Temporal Extent: 2018 - 2023

Dataset Description

This dataset was utilized for Waggoner et al. (submitted). See "Related Datasets" section on this page for other closely-related data from this study published in Waggoner et al. (submitted). They are also listed under the BCO-DMO Project Page: https://www.bco-dmo.org/project/747715.

Methods & Sampling

This data is part of the DOP Hydrolysis Experiments:

Synechococcus Growth– Axenic *Synechococcus* WH8102 (open ocean strain) and WH5701 (coastal strain) were obtained from the National Center for Marine Algae and Microbiota (NCMA, Bigelow Laboratories, East Boothbay, Maine). Both strains were grown in batch culture using SN media (Waterbury *et al.* 1986) made with aged, filtered (0.2 µm), and autoclaved (120°C, 30 minutes) seawater from station ALOHA (A Long-term Oligotrophic Habitat Assessment). At the late-exponential phase, cultures were transferred in triplicate to one of two SN media: (1) +P*i* (45 µmol L^{-1} KH_2PO_4, following Waterbury *et al.* (1986)) and (2) -P*i* (no KH_2PO_4 added; P*i* below detection limit). All cultures were incubated at 25°C on a 12h:12h light cycle at 130 µmol m^{-1} s^{-1} in sterile culture flasks with a vent cap (0.22 µm hydrophobic membrane).

IVF and Flow Cytometry Cell Counts– For each triplicate culture flask and media type (+P and -P), *in vivo* fluorescence (IVF) was measured (AquaFluor®, Turner Designs) as a proxy for *Synechococcus* biomass. In parallel, over the growth curve, 2-mL *Synechococcus* culture aliquots were collected, fixed (final concentration of 0.2% paraformaldehyde), and stored at -80°C until cell abundance analysis using the Guava® EasyCyte flow cytometer (Millipore). Briefly, *Synechococcus* was enumerated in unstained samples based on red fluorescence (*i.e.*, chlorophyll) and forward scatter signals using a low flow rate of 0.24 µL s^{-1} for 1 minute. Instrument-specific beads (Guava® Check Kit, Luminex) were used to calibrate the instrument.

Organism identifiers (Life Science Identifier, LSID):
Synechococcus, urn:lsid:marinespecies.org:taxname:160572

Data Processing Description

DOP hydrolysis rates were normalized to flow cytometry cell counts to account for biomass differences between strains and treatments. The DOP hydrolysis rates can be found in the 'Synechococcus DOP Hydrolysis Experiment - hydrolysis rates' dataset (see "Related Datasets" section).

BCO-DMO Processing Description

* Sheet 1 of submitted file "Synechococcus_DOPHydrolysisExperiment_IVF_CellCounts.xlsx" was imported into the BCO-DMO data system for this dataset. Values "NaN" imported as missing data values.
** Missing data values are displayed differently based on the file format you download. They are blank in csv files, "NaN" in MatLab files, etc.

* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

Problem Description

This dataset is part of the DOP Hydrolysis Experiments. As such, IVF was measured consistently to track the

culture growth curve and flow cytometry cell count samples were taken only on the days when DOP hydrolysis was measured (at each growth phase). As a result, there are more values of IVF than cell counts in the dataset.

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Related Datasets

IsRelatedTo

Duhamel, S., Diaz, J., Waggoner, E., Djaoudi, K. (2024) **Dissolved organic phosphorus (DOP) hydrolysis rates from marine cyanobacterium Synechococcus (WH8102 and WH5701) laboratory cultures from experiments between 2018-2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-05-28 http://lod.bco-dmo.org/id/dataset/928984 [view at BCO-DMO]
Relationship Description: These datasets were collected as part of a study of "Dissolved organic phosphorus bond-class utilization by Synechococcus" (Waggoner et al., submitted).

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Parameters

Parameter	Description	Units
synechococcus_strain	Synechococcus strain. Two were tested, WH8102 and WH5701	unitless
time_day	the day a culture aliquot was taken	days
media_and_phosphate_level	culture was grown in SN media either with phosphate (+P) or without (-P). See methods.	unitless
in_vivo_fluor_trip1	in vivo fluorescence for triplicate flask #1	relative fluorescence units (RFU)
in_vivo_fluor_trip2	in vivo fluorescence for triplicate flask #2	relative fluorescence units (RFU)
in_vivo_fluor_trip3	in vivo fluorescence for triplicate flask #3	relative fluorescence units (RFU)
cell_counts_trip1	Flow cytometry cell counts for triplicate flask #1	cells per milliliter (cells mL-1)
cell_counts_trip2	Flow cytometry cell counts for triplicate flask #2	cells per milliliter (cells mL-1)
cell_counts_trip3	Flow cytometry cell counts for triplicate flask #3	cells per milliliter (cells mL-1)

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Instruments

Dataset-specific Instrument Name	Molecular Devices M2 multimode plate reader (Spectra Max)
Generic Instrument Name	plate reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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Project Information

Collaborative Research: Assessing the role of compound-specific phosphorus hydrolase transformations in the marine phosphorus cycle (P-hydrolase)

NSF Award Abstract:
Phosphorus (P) is an essential building block for life. Because P is in short supply over vast areas of the ocean, P availability may control biological productivity, such as photosynthesis and carbon fixation, which has implications for uptake of the greenhouse gas carbon dioxide and thus climate regulation. Marine microorganisms must satisfy their nutritional requirement for P by obtaining it from seawater, where P is present in a variety of chemical forms, from simple phosphate ions (Pi) to complex dissolved organic phosphorus (DOP) molecules. The concentration of DOP vastly exceeds Pi over most ocean areas, therefore DOP is a critically important source of P for marine microbial nutrition and productivity. However, much remains unknown about the contribution of specific DOP compounds to the P nutrition, productivity, and structure of marine microbial communities. In this project, the investigators will conduct field experiments in the Atlantic Ocean and perform a series of controlled laboratory studies with pure enzymes and microbial cultures to determine how and to what extent different DOP compounds are degraded to Pi in the marine environment. Furthermore, the contribution of these compound-specific DOP molecules to microbial P nutrition, carbon fixation, and community structure will be determined, thus advancing the current state of knowledge regarding the factors that control the activity and distribution of microbial species in the ocean, and the ocean?s role in the climate system. This project will support two female junior investigators, a postdoctoral researcher, and graduate and undergraduate students. The undergraduate students will be recruited from the Marine Sciences program at Savannah State University, an Historically Black Colleges and Universities. In addition, results will be incorporated into new hands-on K-12 educational tools to teach students about microbial P biogeochemistry, including a digital game and formal lesson plans with hands-on demos. These tools will be validated with K-12 educators and will be widely accessible to the public through various well-known online platforms. These activities will thus reach a broad audience including a significant fraction of underrepresented groups.

P is a vital nutrient for life. Marine microorganisms utilize P-hydrolases, such as alkaline phosphatase (AP), to release and acquire phosphate (Pi) from a wide diversity of dissolved organic P (DOP) compounds, including P-esters (P-O-C bonds), phosphonates (P-C), and polyphosphates (P-O-P). Compound-specific DOP transformations have the potential to exert critical and wide-ranging impacts on marine microbial ecology (e.g. variable DOP bioavailability among species), biogeochemistry (e.g. P geologic sequestration via formation of calcium Pi), and global climate (e.g. aerobic production of the greenhouse gas methane by dephosphorylation

of methylphosphonate). However, the mechanisms and comparative magnitude of specific DOP transformations, in addition to their relative contributions to microbial community-level P demand, productivity, and structure, are not completely understood. This study will fill these knowledge gaps by tracking the fate of specific DOP pools in the marine environment. Specifically, this project will test four hypotheses in the laboratory using recombinant enzymes and axenic cultures representative of marine eukaryotic and prokaryotic plankton from high and low nutrient environments, and in the field using observational and experimental approaches along natural Pi gradients in the Atlantic Ocean. In particular, the investigators will reveal potential differences in the hydrolysis and utilization of specific DOP compounds at the community- (bulk enzymatic assays), taxon- (cell sorting of radiolabeled cells in natural samples), species- (axenic cultures) and molecular-levels (pure enzyme kinetic studies and cell-associated proteomes and exoproteomes). Results from our proposed work will provide a robust understanding of the enzymatic basis involved in the transformation of specific forms of DOP and create new knowledge on the relative contribution of these specific P sources to Pi production, marine microbial nutrition, community structure, primary productivity, and thus global carbon cycling and climate. In particular, our refined measurements of the concentration of bioavailable DOP and our unique estimates of DOP remineralization fluxes will provide critical new information to improve models of marine primary production and P cycling.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736967
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