Hydrolysis rates from dissolved organic phosphorus (DOP) hydrolysis experiments with marine cyanobacterium Synechococcus laboratory cultures (WH8102 and WH5701) from 2018-2023

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Data Type: experimental
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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
Dissolved organic phosphorus (DOP) hydrolysis rates from marine cyanobacterium Synechococcus (WH8102 and WH5701) laboratory cultures. These data were collected as part of a study of "Dissolved organic phosphorus bond-class utilization by Synechococcus" (Waggoner et al., 2024). 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. (2024) Figure 2 and supplementary figure 2. See "Related Datasets" section on this page for other closely-related data from this study published in Waggoner et al. (2024). They are also listed under the BCO-DMO Project Page: https://www.bco-dmo.org/project/747715.

Methods & Sampling

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).

DOP Hydrolysis– Representative phosphoester (P-ester) and polyphosphate (PolyP) hydrolysis rates were determined as in Diaz *et al.* (2018). The nucleotide AMP was used as a model P-ester, 3-PolyP as a model PolyP, and ATP as a representative containing both P-ester and P-anhydride bonds. Two separate experiments were conducted with both *Synechococcus* strains to determine DOP hydrolysis in the presence (whole cell) and absence (cell-free filtrate) of cells (Diaz *et al.* 2018, 2019). The +P*i* and -P*i* cultures were subsampled approximately every three days over ~20 days to obtain whole cell and cell-free DOP hydrolysis results along each phase of the cellular growth curve. To prepare cell-free filtrates, the +P*i* and -P*i* culture aliquots were aseptically filtered (0.2 µm) at each subsampling day. To determine DOP hydrolysis rates, the production of P*i* was measured over time. Aliquots (200 µL) of +P*i* and -P*i* treatments were amended with a single DOP substrate (3-PolyP, ATP or AMP; 20 µmol L^{-1} P, final concentration) in triplicate wells of a non-treated standard 96-well transparent microplate and incubated over 6-hours. An unamended control treatment to track P*i* concentrations in the cultures over time were prepared in triplicate and monitored in parallel. Pi (or soluble reactive phosphorus) was measured using a standard colorimetric protocol (Hansen and Koroleff 1999) on a multimode plate reader (SpectraMax® M2, Molecular Devices). Absorbance read at 880 nm was calibrated using a standard curve of monopotassium phosphate (0, 0.5, 1, 2, 5, 10, 20, 40, 50, 75 µmol L^{-1}; KH_2PO_4). The average detection limit of P*i* using this method, defined as three times the standard deviation of the triplicate blank measurements, was 0.125 ± 0.005 µmol L^{-1}. The calibration curve was prepared with 0.2 µm filtered ALOHA seawater with a P*i* background concentration below the detection limit.

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 (can be found in the '*Synechococcus* DOP Hydrolysis Experiment - Cell Counts and IVF' dataset under this project) to account for biomass differences between strains and treatments.

BCO-DMO Processing Description

* Sheet 1 of submitted file "Synechococcus_DOPHydrolysisExperiment_HydrolysisRates.xlsx" was imported into the BCO-DMO data system for this dataset.

* 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]

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Data Files

File
928984_v1_syn-dop-exp-hyrolysis.csv (Comma Separated Values (.csv), 11.01 KB) MD5:292bfc2ad5eae04bace6e758080cb223 Primary data file for dataset ID 928984, version 1

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Related Publications

Waggoner, E. M., Djaoudi, K., Diaz, J. M., & Duhamel, S. (2024). Dissolved Organic Phosphorus Bond-Class Utilization by Synechococcus. FEMS Microbiology Ecology. https://doi.org/10.1093/femsec/fiae099
Results

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Related Datasets

IsRelatedTo

Duhamel, S., Diaz, J., Djaoudi, K., Waggoner, E. (2024) **In vivo fluorescence and flow cytometry cell counts from dissolved organic phosphorus (DOP) hydrolysis experiments with marine cyanobacterium Synechococcus laboratory cultures (WH8102 and WH5701) from 2018-2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-06-06 doi:10.26008/1912/bco-dmo.929471.1 [view at BCO-DMO]
Relationship Description: These datasets were collected as part of the same study published in "Dissolved organic phosphorus bond-class utilization by Synechococcus" (Waggoner et al., 2024, doi: 10.1093/femsec/fiae099).

Duhamel, S., Diaz, J., Djaoudi, K., Waggoner, E. (2024) **Laboratory-cultured Synechococcus (WH8102 and WH5701) MUF-P hydrolysis inhibition by dissolved organic phosphorus (DOP) from experiments between 2018-2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-06-06 doi:10.26008/1912/bco-dmo.929459.1 [view at BCO-DMO]
Relationship Description: These datasets were collected as part of the same study published in "Dissolved organic phosphorus bond-class utilization by Synechococcus" (Waggoner et al., 2024, doi: 10.1093/femsec/fiae099).

Duhamel, S., Diaz, J., Djaoudi, K., Waggoner, E. (2024) **Laboratory-cultured Synechococcus (WH8102 and WH5701) growth (vivo fluorescence) on dissolved organic phosphorus (DOP) from experiments between 2018-2023.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-06-03 doi:10.26008/1912/bco-dmo.929212.1 [view at BCO-DMO]
Relationship Description: These datasets were collected as part of the same study published in "Dissolved organic phosphorus bond-class utilization by Synechococcus" (Waggoner et al., 2024, doi: 10.1093/femsec/fiae099).

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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 to measure DOP hydrolysis rates. Days since T0 of the experiment when the culture was transferred to new media and marked the start of the experiment.	days
sample_type	Sample type description. Two experiments were carried out to measure "whole cell" and "cell-free filtrate" DOP hydrolysis.	unitless
media_and_phosphate_level	culture was grown in SN media either with phosphate (+P) or without (-P)	unitless
DOP_substrate	DOP substrate. "unamended," or on a sampling day, culture was amended with 3-Polyphosphate ("3-PolyP"), adenosine triphosphate ("ATP") or adenosine monophosphate ("AMP") to measure hydrolysis over 6-hrs.	unitless
hydrolysis_rates_trip1	hydrolysis rate for triplicate culture flask #1. 0 indicates no hydrolysis measured.	attomols per cel per hour (amol cell -1 hr -1)
hydrolysis_rates_trip2	hydrolysis rate for triplicate culture flask #2. 0 indicates no hydrolysis measured.	attomols per cel per hour (amol cell -1 hr -1)
hydrolysis_rates_trip3	hydrolysis rate for triplicate culture flask #3. 0 indicates no hydrolysis measured.	attomols per cel per hour (amol cell -1 hr -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
NSF Division of Ocean Sciences (NSF OCE)	OCE-1737083
NSF Division of Ocean Sciences (NSF OCE)	OCE-2001212
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948042

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