Laboratory-cultured Synechococcus (WH8102 and WH5701) growth on dissolved organic phosphorus (DOP) from experiments between 2018-2023

Website: https://www.bco-dmo.org/dataset/929212
Data Type: experimental
Version: 1
Version Date: 2024-06-03

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

Contributors	Affiliation	Role
Duhamel, Solange	University of Arizona (UA)	Principal Investigator
Diaz, Julia	University of California-San Diego (UCSD-SIO)	Co-Principal Investigator
Djaoudi, Kahina	University of Arizona (UA)	Scientist
Waggoner, Emily	University of Arizona (UA)	Scientist
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Laboratory culture Synechococcus (WH8102 and WH5701) growth on dissolved organic phosphorus (DOP). These data were collected as part of 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.

Table of Contents

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) Figure 1. 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

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). 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).

Growth on DOP Substrates – The capacity of *Synechococcus* WH8102 and WH5701 to grow on DOP as a sole phosphorus source was tested in -Pi SN media amended with a single DOP substrate (45 µmol L-1 P, final concentration, Waterbury et al. (1986)). Representative DOP compounds included the P-monoesters glucose-6-phosphate (Glc-6-P) and adenosine 5'-monophosphate (AMP); the P-diester Bis(4-methylumbelliferyl) phosphate (BisMUF-P); the short and long chain polyphosphates: 3-polyphosphate (3-PolyP) and 45-polyphosphate (45-PolyP); the P-monoester and P-anhydride containing adenosine 5'-triphosphate (ATP); and the phosphonates: 4-nitrophenyl phenylphosphonate (4-NpPn), 2-aminomethylphosphonic acid (2-AEPn), methylphosphonic acid (MPn), and ethylphosphonic acid (EPn). Two separate experiments were carried out in triplicate to test growth on 1) P-monoester and PolyP substrates and 2) a P-diester and phosphonates. Two control treatments (culture grown in +Pi and -Pi media) were carried out in triplicate for each experiment. IVF was measured daily in each treatment over ~20 days, and cell axenicity was tested every ~5 days.

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]

[table of contents | back to top]

Related Publications

Waggoner, Emily M; Djaoudi, Kahina; Diaz, Julia M; Duhamel, Solange (submitted). Dissolved Organic Phosphorus Bond-Class Utilization by Synechococcus. FEMS Microbiology Ecology.
Results

Waterbury, J., Watson, S., Valois, F., and Franks, D. (1986). Biological and ecological characterization of the Marine Unicellular Cyanobacterium Synechococcus. Can. Bull. Fish. Aquat. Sci. 214, 71-120.
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
synechococcus_strain	Synechococcus strain. Two were tested, WH8102 and WH5701	unitless
experiment_number	Experiment number (1 or 2). Two experiments were carried out, 1. growth on P-monoesters and PolyP; and 2. growth on P-diester and phosphonates	unitless
time_day	The day a culture aliquot was taken to measure IVF	days
DOP_substrate	DOP substrate added to -P SN media as the sole phosphorus source for culture (see methodology)	unitless
in_vivo_fluorescence_trip1	in vivo fluorescence for triplicate culture flask #1	relative fluorescence units (RFU)
in_vivo_fluorescence_trip2	in vivo fluorescence for triplicate culture flask #2	relative fluorescence units (RFU)
in_vivo_fluorescence_trip3	in vivo fluorescence for triplicate culture flask #3	relative fluorescence units (RFU)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	AquaFluor® Handheld Fluorometer and Turbidimeter (Turner Designs)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	AquaFluor® Handheld Fluorometer and Turbidimeter (Turner Designs)
Generic Instrument Name	Turbidity Meter
Generic Instrument Description	A turbidity meter measures the clarity of a water sample. A beam of light is shown through a water sample. The turbidity, or its converse clarity, is read on a numerical scale. Turbidity determined by this technique is referred to as the nephelometric method from the root meaning "cloudiness". This word is used to form the name of the unit of turbidity, the NTU (Nephelometric Turbidity Unit). The meter reading cannot be used to compare the turbidity of different water samples unless the instrument is calibrated. Description from: http://www.gvsu.edu/wri/education/instructor-s-manual-turbidity-10.htm (One example is the Orion AQ4500 Turbidimeter)

[table of contents | back to top]

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.

[table of contents | back to top]

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

[table of contents | back to top]