Dissolved organic phosphorus (DOP) hydrolysis rates from Ruegeria pomeroyi laboratory cultures

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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
Diaz, Julia	University of California-San Diego (UCSD-SIO)	Principal Investigator
Duhamel, Solange	University of Arizona (UA)	Co-Principal Investigator
Adams, Jamee	University of California-San Diego (UCSD-SIO)	Scientist
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
Dissolved organic phosphorus (DOP) hydrolysis rates from marine bacterium Ruegeria pomeroyi laboratory cultures. These data were collected as part of a study of "Dissolved organic phosphorus utilization by the marine bacterium Ruegeria pomeroyi DSS-3 reveals chain length-dependent polyphosphate degradation" (Adams et al., 2022). Study abstract: Dissolved organic phosphorus (DOP) is a critical nutritional resource for marine microbial communities. However, the relative bioavailability of different types of DOP, such as phosphomonoesters (P-O-C) and phosphoanhydrides (P-O-P), is poorly understood. Here we assess the utilization of these P sources by a representative bacterial copiotroph, Ruegeria pomeroyi DSS-3. All DOP sources supported equivalent growth by R. pomeroyi, and all DOP hydrolysis rates were upregulated under phosphorus depletion (-P). A long-chain polyphosphate (45polyP) showed the lowest hydrolysis rate of all DOP substrates tested, including tripolyphosphate (3polyP). Yet the upregulation of 45polyP hydrolysis under -P was greater than any other substrate analyzed. Proteomics revealed three common P acquisition enzymes potentially involved in polyphosphate utilization, including two alkaline phosphatases, PhoD and PhoX, and one 5'-nucleotidase (5'-NT). Results from DOP substrate competition experiments show that these enzymes likely have broad substrate specificities, including chain length-dependent reactivity toward polyphosphate. These results confirm that DOP, including polyP, are bioavailable nutritional P sources for R. pomeroyi, and possibly other marine heterotrophic bacteria. Furthermore, the chain-length dependent mechanisms, rates and regulation of polyP hydrolysis suggest that these processes may influence the composition of DOP and the overall recycling of nutrients within marine dissolved organic matter.

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Coverage

Temporal Extent: 2021-03-09 - 2021-03-17

Methods & Sampling

Experimental Procedures

Culture conditions and growth tracking

R. pomeroyi DSS-3 was cultured in media modified from the recipe of Rivers et al. (2016). Briefly, media (100 mL) were prepared using 0.2 µm-filtered natural seawater collected from the Scripps Institution of Oceanography pier that was autoclaved (121°C, 20 min) in 125 mL acid-washed glass Erlenmeyer flasks. Sterile-filtered (0.2 µm) glucose and nutrient stocks, including P sources, were aseptically added to the sterile seawater base in a laminar flow hood. Phosphate-replete media (+Pi) contained 18 µM P. P depleted media (-P) were prepared by adding phosphate to a final concentration of 1.8 µM P. ATP (Millipore Sigma), AMP (Fisher Scientific), 3polyP (Millipore Sigma), or 45polyP (Millipore Sigma) were added to -P media at a final concentration of 18 µM P. All media were inoculated with 50 µL of R. pomeroyi grown to stationary phase in +Pi media, in order to limit the carryover of P. Cultures were grown in a Thermo shaker/incubator at 30˚C with shaking at 150rpm for 10 days. Samples for optical density (600 nm) and flow cytometry were taken daily. Flow cytometry samples were prepared by sampling 2ml of cultures into cryovials, preserved with a final concentration of 0.5% glutaraldehyde at 4˚C for 10 minutes, and frozen at -80°C until analysis. Growth rates were calculated over the interval of log-linear growth in +Pi cultures. Growth rates in -P cultures were calculated over the same time period as +Pi cultures. All growth experiments were performed in triplicate.

DOP hydrolysis

DOP hydrolysis rates were determined by following the production of Pi, as described previously (Diaz et al., 2018, 2019). R. pomeroyi culture samples were collected at the same time from both +Pi and -P media, according to the time period of midlog and stationary phases for +Pi cultures. Two types of samples were prepared from each culture. First, whole culture samples were diluted 1:10 or 1:20 with sterile-filtered (0.2 µm) natural seawater (Diaz et al., 2019). Second, cultures were filtered (0.22 µm) in order to generate the cell-free filtrates. Diluted whole culture and filtrate samples were then added to clear 96-well plates and amended with the DOP sources ATP, AMP, 3polyP, 45polyP, or MUF-P (18 µM, final molecular concentration) or Pi (18 µM, final). Briefly, Pi was quantified as soluble reactive phosphorus (SRP) following the method of Hansen and Koroleff (1999) using a multimode plate reader (Molecular Devices) with a detection limit of 800 nmol L-1 P (Diaz et al., 2018). Samples were reacted at 4 to 6 specific timepoints up to 24 hr. Each Pi measurement that was derived from a diluted whole culture sample was corrected for cellular Pi uptake as described previously (Diaz et al., 2019), and hydrolysis rates were calculated as the slope of Pi production over time using a simple linear regression (typically R2 > 0.95). Hydrolysis rates in diluted whole culture samples were corrected for dilution after regression analysis. To assess abiotic DOP hydrolysis, culture samples were filtered (0.2 µm) and boiled (99°C, 15 min). DOP hydrolysis in these controls was negligible.

APA competition plates

APA was assessed at midlog and stationary phases in undiluted whole culture samples and cell-free filtrates in the presence and absence of DOP sources by following hydrolysis of the fluorogenic substrate MUF-P (Millipore Sigma). MUF-P hydrolysis was tracked using a multimode plate reader (Molecular Devices), using 359 nm and 449 nm as the excitation and emission wavelengths, respectively. Final molecular concentrations of DOP substrates were 0-100 µM (Pi as a control, ATP, AMP, 3polyP, and 45polyP). The final concentration of MUF-P was 10 µM. Experiments were run in kinetic mode for 15 minutes collecting data every 30 seconds. Enzyme activity was calculated as a percentage of the control hydrolysis rate (no DOP added) to illustrate inhibition of MUF-P by the unlabeled DOP substrates. IC50 values for each inhibiting DOP substrate were calculated from the linear regression of log normalized substrate concentrations versus the percent inhibition of MUF-P by each substrate (R2 typically >0.90). IC50 values were calculated according to the formula: IC50 = (0.5-b)/a, where b is the y-intercept and a is the slope of the linear regression.

Flow Cytometry (see Related dataset " Ruegeria pomeroyi OD and FCM" https://www.bco-dmo.org/dataset/897371).

Taxonomic Identifiers (Species, LSID):
Ruegeria pomeroyi, urn:lsid:marinespecies.org:taxname:567965

Data Processing Description

BCO-DMO Data Processing Notes:

* Excel file "R. pom hydrolysis rates_BCO-DMO.xlsx" loaded into the bco-dmo data system.
* Column names modified to match BCO-DMO naming conventions to support broad interoperability.

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Data Files

File	
dop-hydrolysis-rates.csv	(Octet Stream, 4.72 KB) MD5:0637aa894d0f1517bc1cbb657c665b7d
Primary data table for dataset 897359.	

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Related Publications

Adams, J. C., Steffen, R., Chou, C., Duhamel, S., & Diaz, J. M. (2022). Dissolved organic phosphorus utilization by the marine bacterium Ruegeria pomeroyi DSS-3 reveals chain length-dependent polyphosphate degradation. Environmental Microbiology, 24(5), 2259–2269. Portico. https://doi.org/10.1111/1462-2920.15877
Results

Diaz, J. M., Holland, A., Sanders, J. G., Bulski, K., Mollett, D., Chou, C.-W., ... Duhamel, S. (2018). Dissolved Organic Phosphorus Utilization by Phytoplankton Reveals Preferential Degradation of Polyphosphates Over Phosphomonoesters. Frontiers in Marine Science, 5. doi:10.3389/fmars.2018.00380
Methods

Diaz, J. M., Steffen, R., Sanders, J. G., Tang, Y., & Duhamel, S. (2019). Preferential utilization of inorganic polyphosphate over other bioavailable phosphorus sources by the model diatoms Thalassiosira spp. Environmental Microbiology, 21(7), 2415–2425. Portico. https://doi.org/10.1111/1462-2920.14630
Methods

Rivers, A. R., Smith, C. B., & Moran, M. A. (2014). An Updated genome annotation for the model marine bacterium Ruegeria pomeroyi DSS-3. Standards in Genomic Sciences, 9(1). https://doi.org/10.1186/1944-3277-9-11
Methods

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Related Datasets

IsRelatedTo

Diaz, J., Duhamel, S., Adams, J. (2023) **Optical density and cell counts from flow cytometry of Ruegeria pomeroyi laboratory cultures.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2023-06-09 doi:10.26008/1912/bco-dmo.897371.1 [view at BCO-DMO]
Relationship Description: Used the same Ruegeria pomeroyi cultures.

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Parameters

Parameter	Description	Units
Media_Type	Media type used in culture	unitless
Growth_Phase	Growth phase (e.g. Statinary phase, Midlog)	unitless
Sample_Type	Sample type (e.g. Filtrate, Whole cell)	unitless
DOP_Substrate	dissolved organic phosphorus substrate	unitless
Hydrolysis_Rate	Hydrolysis rate	umol Pi L-1 hr-1

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Instruments

Dataset-specific Instrument Name	Molecular Devices M3 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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NSF Division of Ocean Sciences (NSF OCE)	OCE-2001212
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948042

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