Optical density and cell counts from flow cytometry of Ruegeria pomeroyi laboratory cultures

Website: https://www.bco-dmo.org/dataset/897371
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
Optical density (OD) and cell counts from flow cytometry (FCM) of 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 - 2021-03-23

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 and APA competition plates (see related "Ruegeria pomeroyi DOP hydrolysis rates" dataset https://www.bco-dmo.org/dataset/897359)

Flow Cytometry

For cell counts, culture samples were preserved in filtered (0.22 µm) glutaraldehyde (0.5% final concentration), left to fix at 4˚C for 10 minutes, and frozen at -80°C until analysis. Preserved samples were thawed and counted on a Guava EasyCyte HT flow cytometer (Millipore), and instrument calibration was performed using instrument-specific beads (Luminex). Prior to running on the flow cytometer, samples were prepared in clear, round-bottom 96 well plates (Fisher Scientific) and diluted with filtered (0.22 µm) seawater either 100X (T0, T1) or 1000X (T2 - T8). Triplicate blanks prepared with filtered (0.22 µm) seawater and glutaraldehyde (0.5% final concentration) were run with samples, and the average blank cell count was subtracted from all samples. Blanks and diluted samples were stained with diluted SYBR Green nucleic acid gel stain (diluted in deionized water to 100X; Fisher Scientific) and left in the dark for 30 minutes. After staining, bacterial cell concentrations were analyzed at a low flow rate (0.24 µL s-1) for 3 minutes, and cells were counted based on diagnostic forward scatter versus green fluorescence signals.

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

Time ranges: Experiments were performed 3/9/21 - 3/17/21. Fixed flow cytometry samples were run from 3/18/21 - 3/23/21

Data Processing Description

BCO-DMO Data Processing Notes:
* Excel file "R. pom OD & FCM_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	
od-fcm.csv	(Octet Stream, 4.52 KB)
	MD5:89bff5c6697ab5e6fd5dba45fdc96bbc
Primary data table for dataset 897371.	

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Supplemental Files

File
Ruegeria pomeroyi FCS files filename: Ruegeria_pomeroyi_FCS_files.zip (Octet Stream, 226.65 MB) MD5:833207c2cc448c0094e78deb86b269a8 Ruegeria pomeroyi flow cytometry .fcs files (Flow Cytometry Standard files). Zip package contains 11 .fcs files (e.g. "R. pom FCM_2021-03-18_T0.fcs"). Analyzed volume: 200uL Data producer: Jamee Adams Fixed: 0.5% final concentration Glutaraldehyde, frozen at -80˚C until analysis Stained: SYBR green Dilution: 1:1000 Prefiltration: n/a

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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., Adams, J., Duhamel, S. (2023) **Dissolved organic phosphorus (DOP) hydrolysis rates from 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.897359.1 [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_Day	Growth day (numeric, 0..n).	unitless
Optical_Density	Optical Density (OD)	optical_density
FCM	Flow Cytometry cell concentration	cells per milliliter (cells/ml)

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Instruments

Dataset-specific Instrument Name	Guava EasyCyte flow cytometer (Luminex)
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)

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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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