Measurements of C–P lyase phosphate thresholds in Stutzerimonas frequens cultures (North Pacific Methane Project)

Website: https://www.bco-dmo.org/dataset/984527
Data Type: experimental
Version: 1
Version Date: 2025-09-18

Project
» Collaborative Research: NSF OCE-BSF: Coupling organic nutrient cycling to methane production in the oligotrophic North Pacific Ocean (North Pacific methane)

Contributors	Affiliation	Role
Repeta, Daniel J.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
Sosa, Oscar A.	University of Puget Sound	Co-Principal Investigator
Bargman, Claire	University of Puget Sound	Scientist
Björkman, Karin	University of Hawai'i (UH)	Scientist
Foreman, Rhea	University of Hawai'i (UH)	Scientist
Granzow, Benjamin	University of California-San Diego (UCSD)	Scientist
Karl, David M.	University of Hawai'i (UH)	Scientist
Rudolph, Xander	University of Puget Sound	Scientist
Newman, Sawyer	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset contains results from an experiment investigating the phosphate concentration threshold for C-P lyase activity in cultures of Stutzerimonas frequens. The dataset includes measurements of total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), and dissolved organic phosphorus (DOP) in the culture medium at the end of incubation. Additional parameters include cell concentrations from cultures, methane produced by the cultures during the experiment, and rates of C-P lyase activity (CLA). TDP, SRP, and DOP were measured using standard colorimetric methods. Cell concentrations were determined by flow cytometry. Methane concentrations and $\delta^{13}C$ values were measured with a Picarro gas analyzer, and CLA was quantified using a fluorescently labeled phosphonate probe. These data demonstrate that C-P lyase activity is regulated by dissolved phosphate concentrations and that S. frequens upregulates C-P lyase genes when phosphate concentrations drop below 90 nM. The cultures were grown by Dr. Oscar Sosa, and the samples were analyzed by Dr. Benjamin Granzow.

Table of Contents

Coverage

Temporal Extent: 2024-06 - 2024-06

Methods & Sampling

Cultures of *S. frequens* were grown in 150 mL of seawater medium in acid-clean, sterilized glass serum bottles. The medium was amended with methylphosphonate to a final concentration of 73.5 nM, and the fluorescently labeled phosphonate tracer n-DPPh (Naphthyl-diphenylphosphonate) was added to the medium to a final concentration of 7.4 nM. Cultures were incubated for 4 days.

At the end of incubation, samples were collected for chemical and cellular analyses. 10 mL of headspace gas were withdrawn via an airtight syringe for methane concentration and $\delta^{13}C$ isotope analysis using a Picarro gas analyzer. 50 mL of culture medium were collected for phosphorus analysis using standard colormetric methods (Murphy et al.,1962). 1 mL of medium was collected for cell counts via flow cytometry. Finally, 10 mL of medium were collected for C-P lyase activity analysis by HPLC coupled with a fluorescence detector.

Data Processing Description

All data were processed in R (version 4.4.3) within RStudio (version 2024.12.0+467) using a custom peak-finding and area-calculation script (not published). The script incorporates functions from several standard R packages, including tidyverse, signal, PROcess, and pracma.

- R Core Team. (2024). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. Version 4.4.3. Retrieved from https://www.r-project.org/
- Posit Software, PBC. (2024). *RStudio: Integrated development for R.* Version 2024.12.0+467. Retrieved from http://www.rstudio.com/
- Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. (2019). *tidyverse: Easily install and load the tidyverse.* R package. Version 2.0.0. Retrieved from https://doi.org/10.21105/joss.01686
- Signal Developers. (2023). *signal: Signal processing.* R package. Version X.X. Retrieved from https://r-forge.r-project.org/projects/signal/
- PROcess Developers. (2023). *PROcess: LC-MS preprocessing and analysis pipeline.* R package. Version X.X. Retrieved from https://doi.org/10.18129/B9.bioc.PROcess
- Borchers H. W. (2023). *pracma: Practical numerical math functions.* R package. Version 2.4.4. Retrieved from https://CRAN.R-project.org/package=pracma

BCO-DMO Processing Description

- Within the primary data file (984527_v1_cla_phosphate_threshold_data.csv), parameter names were altered to conform to FAIR data standards. Special characters and blank spaces were replaced with underscores ("_"). Units were also removed from parameter names, and can be found within the parameter details section of the metadata record.
- Missing data values in numeric/float fields were originally represented as "-999" values; these have been replaced with blank data values.

[table of contents | back to top]

Data Files

File	
984527_v1_cla_phosphate_threshold_data.csv	(Comma Separated Values (.csv), 5.29 KB) MD5:1ee0fdb4b5f54533611554533db4ab9d
Primary data file for dataset ID 984527, version 1	

[table of contents | back to top]

Supplemental Files

File	
984527_culture_condition_description.csv	(Comma Separated Values (.csv), 585 bytes) MD5:bd0eb6fc2f618d883abb5bf5e26270aa
Detailed description of the different culturing conditions represented in primary data file (984527_v1_cla_phosphate_threshold_data.csv).	

[table of contents | back to top]

Related Publications

Granzow, B. N., Sosa, O. A., Gonnelli, M., Santinelli, C., Karl, D. M., & Repeta, D. J. (2021). A sensitive fluorescent assay for measuring carbon-phosphorus lyase activity in aquatic systems. Limnology and Oceanography: Methods, 19(4), 235–244. Portico. https://doi.org/10.1002/lom3.10418
Methods

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta, 27, 31–36. doi:10.1016/s0003-2670(00)88444-5
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Bottle	Bottle number from culture experiment.	unitless
Condition	Culture condition, which includes variation in initial phosphate concentration, fluorescent phosphonate probe, and bacterial inoculation. See Culture_condition_description.csv for specific details.	unitless
Type	Sample type (control, blank, sample).	unitless
Initial_PO4	Initial phosphate concentration at the beginning of the incubation.	nM
Residual_PO4	SRP in the medium after the incubation.	nM
PO4_Uptake	Amount of phosphate taken up by the bacteria (Initial - Residual).	nM
Initial_TDP	Total dissolved phosphorus concentration at the beginning of the incubation.	nM
TDP	TDP at the end of the incubation.	nM
DOP	DOP at the end of the incubation (TDP - Residual PO4).	nM
cells_ml_avg	Average cell concentration taken from multiple flow cytometry runs.	cells/mL
cells_ml_sd	Standard deviation of cell concentrations taken from multiple flow cytometry runs.	cells/mL
cells_ml_cv	Coefficient of variation of cell concentrations taken from multiple flow cytometry runs.	cells/mL
CH4	Concentration of methane in the headspace and culture medium.	nM
delta13_CH4	delta_13C isotope value of the methane measured by the Picarro gas analyzer.	per_mille
nDPPh_Concentration	n-DPPh concentration at the end of the incubation.	pM
nDP_Concentration	Concentration of nDP, a fluorescent compound produced when C–P lyase cleaves the phosphonate group from the tracer nDPPh. Reported as the concentration present at the end of the incubation, used together with nDPPh measurements to quantify C–P lyase activity (CLA).	pM

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	GUAVA EasyCyte
Generic Instrument Name	Flow Cytometer
Dataset-specific Description	Cell concentrations were determined by flow cytometry using a Guava EasyCyte system on 1 mL aliquots of culture medium.
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)

Dataset-specific Instrument Name	Agilent 1200
Generic Instrument Name	High-Performance Liquid Chromatograph
Dataset-specific Description	C–P lyase activity was quantified from 10 mL of culture medium using high-performance liquid chromatography (HPLC) with fluorescence detection on an Agilent 1200 system.
Generic Instrument Description	A High-performance liquid chromatograph (HPLC) is a type of liquid chromatography used to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of the mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by high pressure pumping of the sample mixture onto a column packed with microspheres coated with the stationary phase. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.

Dataset-specific Instrument Name	Picarro G2201-i
Generic Instrument Name	Picarro G2201-i isotope analyzer
Dataset-specific Description	Methane concentrations and δ^{13}C-CH_4 values were measured using a Picarro G2201-i cavity ring-down spectrometer.
Generic Instrument Description	The G2201-i Isotopic Analyzer measures d13C for CH4 and CO2. See: https://www.picarro.com/products/g2201i_isotopic_analyzer

Dataset-specific Instrument Name	SEAL Analytical AA3
Generic Instrument Name	Seal Analytical AutoAnalyser 3HR
Dataset-specific Description	Phosphorus concentrations were measured on 50 mL aliquots of culture medium using standard colorimetric methods with a SEAL Analytical AA3 autoanalyzer.
Generic Instrument Description	A fully automated Segmented Flow Analysis (SFA) system, ideal for water and seawater analysis. It comprises a modular system which integrates an autosampler, peristaltic pump, chemistry manifold and detector. The sample and reagents are pumped continuously through the chemistry manifold, and air bubbles are introduced at regular intervals forming reaction segments which are mixed using glass coils. The AA3 uses segmented flow analysis principles to reduce inter-sample dispersion, and can analyse up to 100 samples per hour using stable LED light sources.

[table of contents | back to top]

Project Information

Collaborative Research: NSF OCE-BSF: Coupling organic nutrient cycling to methane production in the oligotrophic North Pacific Ocean (North Pacific methane)

Coverage: Oligotrophic gyre surface waters

NSF abstract:

Open ocean surface waters are natural sources of methane to the atmosphere. As recently as a decade ago the source of this methane was a mystery, because methane production was only known to occur in certain environments without oxygen. Recently, the discovery of several metabolic pathways that enable microbes to transform organic matter into methane in the presence of oxygen has led to a shift away from the idea that methane can only be produced in anaerobic (oxygen-free) environments. The investigators propose that the pathway microbes use to make methane depends on the nutrient conditions that prevail in open ocean surface waters. In the North Atlantic Ocean, phosphorus limits microbial production, and microbes produce methane as a by-product of getting the phosphorus they need from organic compounds that contain phosphorus. In contrast, nitrogen limits microbial production in the North Pacific Ocean. The team proposes that in the North Pacific Ocean microbes produce methane as a by-product of organic nitrogen degradation. To test this hypothesis, they propose to compare the results of geochemical and biological measurements previously made in the North Atlantic with a parallel set of geochemical measurements they propose to make in the North Pacific Ocean. The award will support collaborations between an early career professor at a primarily undergraduate institution (PUI) and a senior scientist, and between US and Israeli scientists. Undergraduate students will participate in interdisciplinary research spanning oceanography, isotope biogeochemistry, and genome science and will conduct research at sea. The microbiology and genomic research will be integrated into course-based undergraduate research experiences at the University of Puget Sound enabling diverse students to participate directly in authentic research. Results will also be integrated into a graduate level course in marine organic geochemistry available on-line through the MIT Open Courseware website. This is a project jointly funded by the National Science Foundation's Directorate of Geosciences (NSF-GEO) and the Israel Binational Science Foundation (BSF) in accord with the language in the Memorandum of Understanding between the NSF and the BSF. This Agreement allows a single collaborative proposal, involving US and Israeli investigators, to be submitted and peer-reviewed by NSF. Upon successful results of the NSF merit review and recommendation by the cognizant NSF Program of an award, each Agency funds the proportion of the budget and the investigators associated with its own country.

The guiding hypothesis of this study is that although surface seawater in the North Atlantic and North Pacific Subtropical Gyres are both sources of methane to the atmosphere, the underlying microbial processes that produce methane in the two basins are fundamentally different. Microbial production in the Sargasso Sea is

chronically phosphorus-limited. To mitigate this limitation, some microbes degrade methylphosphonate that is incorporated into the high molecular weight fraction of dissolved organic matter (HMWDOM) into methane and phosphaote. Bacteria expressing the carbon-phosphorus (C-P) lyase enzyme pathway for phosphonate catabolism dominate the Sargasso Sea microbial community and mediate this form of methane production making it the principal route through which excess methane is produced in the Sargasso Sea. In contrast, microbial production in the North Pacific Subtropical Gyre (NPSG) is chronically nitrogen limited and the proposal postulates that nitrogen acquisition through the degradation of methylamines in HMWDOM is a major route through which excess methane is produced. Methylamines are twenty-fold more abundant than methylphosphonate in marine HMWDOM and the aminotransferase gene linked to the conversion of methylamine into methane in freshwater lakes has closely related sequences in marine bacterial genomes. These sequences are abundant and widespread in marine metagenomes. Although the cycling of methylphosphonate and methylamine in oligotrophic surface waters both produce methane, the study postulates that the two processes will yield methane with distinct and characteristic carbon isotopic values. To test this hypothesis, the team will measure the stable carbon isotope value of the methane produced from HMWDOM methylamine and methylphosphonate. The team will also conduct laboratory experiments that test the capacity of diverse oligotrophic and copiotrophic marine bacterial isolates to convert HMWDOM methylamines to methane. This objective is complemented by a field study in the NPSG northwards from Hawaii along 158°W, the longitude of Station ALOHA, to 25-28°N to conduct geochemical and biological measurements associated with each methane production pathway. The team will obtain water column profiles of methane and ethylene concentration (two products of C-P lyase), methane carbon isotopes, and concentrations and carbon isotope values of HMWDOM methylamine and methylphosphonate. The investigators will quantify the rates of methane production from methylamine and methylphosphonate using stable carbon isotope tracers, C-P lyase activity, and the ratio of C-P lyase to aminotransferase gene abundance and expression in the NPSG. Lastly, the team will compare the bioavailability of HMWDOM methylamine and methylphosphonate to natural microbial communities in the NPSG using a metatranscriptomics approach to examine changes in microbial metabolic functions in response to HMWDOM additions. Together, these data will resolve the relative contribution of the methylamine and methylphosphonate pathways to aerobic methane production in the NPSG and the microbial groups and ecosystem properties underlying methane production. Through this interdisciplinary approach, the study will enhance our understanding of processes controlling aerobic methane production in the environment.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2241668
NSF Division of Ocean Sciences (NSF OCE)	OCE-2241667

[table of contents | back to top]