Prochlorococcus or Synechococcus cell concentrations and nitrite concentrations during batch culture with ammonium or nitrate as the sole nitrogen source for growth

Website: https://www.bco-dmo.org/dataset/890887
Data Type: experimental
Version: 1
Version Date: 2023-03-01

Project
» Features and implications of nitrogen assimilation trait variability in populations of Prochlorococcus (Prochlorococcus N assimilation)

Contributors	Affiliation	Role
Berube, Paul	Massachusetts Institute of Technology (MIT)	Principal Investigator
Chisholm, Sallie W.	Massachusetts Institute of Technology (MIT)	Co-Principal Investigator
O'Keefe, Tyler	Massachusetts Institute of Technology (MIT)	Technician
Rasmussen, Anna	Massachusetts Institute of Technology (MIT)	Technician
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
These data include picocyanobacteria (Prochlorococcus or Synechococcus) cell concentrations and nitrite (NO2-) concentrations during batch culture with ammonium (NH4+) or nitrate (NO3-) as the sole nitrogen source for growth. The study was focused on assessing the potential for picocyanobacteria to release nitrite during growth on nitrate due to incomplete assimilatory nitrate reduction. Both pure cultures and co-cultures were assessed.

Table of Contents

Coverage

Temporal Extent: 2019-03 - 2020-02

Methods & Sampling

Cultures: The strains used in this study included *Prochlorococcus* MIT0915, *Prochlorococcus* MIT0917, *Prochlorococcus* MIT1214, *Prochlorococcus* SB, *Synechococcus* WH7803, and *Synechococcus* WH8102. Except for MIT1214, all strains can grow on nitrate as the sole nitrogen source. MIT1214 can use nitrite, but not nitrate. All strains were routinely assayed for heterotrophic contaminants by staining cells with SYBR green and assessing the fluorescence and light scattering properties of both stained and unstained cells using a Guava easyCyte 12HT Flow Cytometer (MilliporeSigma, Burlington, MA, USA). Cultures that did not exhibit the presence of non-photosynthetic cells in the stained samples and had a single cyanobacteria population were presumed axenic and unialgal. All axenic cultures were routinely assessed for purity by confirming a lack of turbidity after inoculation into a panel of purity test broths as described previously (Berube et al., 2015).

Cultivation methods for pure cultures: The cultures were grown on Pro99 medium (Moore et al., 2007) with the sole nitrogen source provided as one of the following: 800 micromolar (µM) ammonium chloride (NH_4Cl), 800 µM sodium nitrate ($NaNO_3$), or 100 µM sodium nitrite ($NaNO_2$). Surface water from the Sargasso Sea was used as the natural seawater base for the Pro99 medium. The cultures were grown as duplicates or triplicates in 35 milliliters (mL) of medium in borosilicate glass culture tubes at a temperature of 24° Celsisus (C) under a range of light intensities under continuous illumination of white or blue light. Cultures were acclimated to each condition for at least 10 generations prior to starting the experiment.

Cultivation methods for co-cultures: MIT1214 was co-cultured with either MIT0915 or MIT0917 in 35 mL of Pro99 medium in borosilicate glass culture tubes with 800 µM of sodium nitrate as the sole nitrogen (N) source. The temperature and light conditions were 24°C and 16 micromoles photons per square meter per second (µmol photons m^{-2} s^{-1}) of continuous blue light, respectively. The co-cultures were grown as triplicates and followed over 2 sequential transfers.

Sampling: Pure cultures were monitored daily by removing 0.5 mL of culture in order to determine total cell concentrations with flow cytometry and nitrite concentrations with the Griess colorimetric method. Co-cultures were treated the same as the pure cultures with sampling for quantitative PCR by filtering 1 mL of culture onto a 25 millimeter (mm) 0.2 micrometer (µm) pore size polycarbonate filter under low vacuum, chasing with 2 mL of qPCR preservation solution (10 millimolar (mM) Tris pH=8, 100 mM EDTA, and 500 mM NaCl), and then transferring the filter to a 2 mL beadbeater tube prior to storage at -80°C.

Nitrite concentration assay: Extracellular nitrite concentrations were determined via the Greiss colorimetric method that uses sequential additions of sulfanilamide and N-(1-naphthyl)ethylenediamine (NED) to produce a pink azo dye with a maximum absorption at a wavelength of 540 nanometers (nm). The following two solutions were prepared: (1) 0.010 grams per milliliter (g/mL) sulfanilamide in 0.6N HCl and (2) 0.001 g/mL NED. These reagent solutions were filtered through a 0.2 µm filter into UV-resistant bottles and stored at 4°C for up to one month. Aliquots of a 1 mM sodium nitrite ($NaNO_2$) standard solution was stored frozen at -20°C and thawed daily to prepare dilutions spanning 1-50 µM for the generation of a standard curve. To prepare samples for quantification of nitrite, a 0.15 mL aliquot of each culture was filtered through a 96-well 0.45 µm MultiScreenHTS HVfilter plate (MilliporeSigma, Burlington, MA, USA) capable of capturing >99% of cyanobacteria cells. Dilutions of the sodium nitrite standard were filtered in the same plate as the culture samples to ensure similar treatment. 100 microliters (µL) of filtrate was then transferred from each well to a flat-bottomed, 96-well microplate. The sulfanilamide reagent solution (50 µL) was added to each well, mixed by pipetting, and incubated in the dark for 10 minutes to allow for chromophore formation. Subsequently, The NED reagent solution (50 µL) was added to each well, mixed by pipetting, and incubated in the dark for an additional 10 minutes to allow for coupling and color development. Absorbance at 540 nm was then determined by using a Synergy 2 Plate Reader (BioTek Instruments, Winooski, VT, USA).

Total cell concentrations: Cell concentrations inclusive of total cyanobacteria cells in each culture were obtained by flow cytometry using a Guava easyCyte 12HT Flow Cytometer (MilliporeSigma, Burlington, MA, USA). *Prochlorococcus* and *Synechococcus* cells were detected based on the fluorescence of cellular pigments excited by a 488 nm laser. Cultures were first diluted to between 50 and 500 cells/µL and data were acquired for up to 6 min at a flow rate of 0.024 microliters per second (µL/s). Bead standards (Guava easyCheck beads; MilliporeSigma, Burlington, MA, USA), were run daily to confirm that the instrument was operating within normal parameters and within predefined tolerances for concentrations, scatter, and emission intensity of the beads.

Strain-specific cell concentrations: For MIT0915 and MIT0917, we used a previously developed quantitative PCR assay (Berube et al., 2016). For the detection of MIT1214 we designed quantitative PCR primers targeting the wcaK gene: MIT1214_wcaK_283F (5'-GACTACTGCATTTTCGCTGGG-3') and MIT1214_wcaK_402R (5'- ACCTTCAAAACCTCCAACACC). Samples used to generate standard curves were acquired by growing MIT0915, MIT0917, and MIT1214 to late-exponential phase (approximately 8 x 107 cells mL^{-1}), filtering 5 mL of culture onto a 25 mm 0.2 µm pore size polycarbonate filter under low vacuum, chasing with 3 mL of qPCR preservation solution (10 mM Tris pH=8, 100 mM EDTA, and 500 mM NaCl), and then transferring the filter to a 2 mL beadbeater tube prior to storage at -80°C. Cell concentrations for each culture, at the time of sample filtration, were obtained by flow cytometry. Templates for both experimental cultures and standards were generated by thawing the filters on ice for 2 minutes, adding 650 µL of 10 mM Tris pH=8, and then beadbeating at 4800 rotations per minute (rpm) for 2 minutes. Following beadbeating to remove cells from the filter, 500 µl of the buffer was transferred to a 1.5 mL centrifuge tube and heated at 95°C for 15 minutes to lyse cells. Templates for standard curves were generated by first diluting the resulting template solution to 5.4 x 105 cell equivalents µL-1 and then performing a serial dilution. All templates were stored at -80°C until use. The MIT1214 *wca*K assay was performed in 25 µL reaction volumes with 2.5 µL template and the following final concentrations of reaction components: 12.5 µL QuantiTect SYBR Green PCR Mix (Qiagen, Germantown, Maryland) and 0.5 µmol L^{-1} of each forward and reverse primer. Using a CFX96 Thermocycler

(Bio-Rad, Hercules, CA, USA), reactions were pre-incubated at 95°C for 15 minutes to activate the polymerase and then cycled (40 cycles) at 95°C for 15 seconds (s), 57°C for 30 s, and 72°C for 30 s. The MIT0915 and MIT0917 *nar*B assays were performed similarly, except for annealing at 60°C for 30 s (Berube et al., 2016). Amplification efficiencies were 85% for the MIT1214 *wca*K assay, 90% for the MIT0915 *nar*B assay, and 79% for the MIT0917 *nar*B assay. Negative controls included MIT0915 and MIT0917 templates for the MIT1214 *wca*K assay as well as MIT1214 templates for the *nar*B assay; no amplification was observed in these negative controls.

[table of contents | back to top]

Data Files

File
cells_nitrate_data.csv (Comma Separated Values (.csv), 133.60 KB) MD5:685c17e75a4be586e8f21a7c85b53019 Primary data file for dataset ID 890887

[table of contents | back to top]

Related Publications

Berube, P. M., Biller, S. J., Kent, A. G., Berta-Thompson, J. W., Roggensack, S. E., Roache-Johnson, K. H., Ackerman, M., Moore, L. R., Meisel, J. D., Sher, D., Thompson, L. R., Campbell, L., Martiny, A. C., & Chisholm, S. W. (2014). Physiology and evolution of nitrate acquisition in Prochlorococcus. The ISME Journal, 9(5), 1195–1207. https://doi.org/10.1038/ismej.2014.211
Methods

Berube, P. M., Coe, A., Roggensack, S. E., & Chisholm, S. W. (2015). Temporal dynamics of Prochlorococcuscells with the potential for nitrate assimilation in the subtropical Atlantic and Pacific oceans. Limnology and Oceanography, 61(2), 482–495. doi:10.1002/lno.10226
Methods

Moore, L. R., Coe, A., Zinser, E. R., Saito, M. A., Sullivan, M. B., Lindell, D., Frois-Moniz, K., Waterbury, J., & Chisholm, S. W. (2007). Culturing the marine cyanobacterium Prochlorococcus. Limnology and Oceanography: Methods, 5(10), 353–362. Portico. https://doi.org/10.4319/lom.2007.5.353
Methods

[table of contents | back to top]

Parameters

Parameter	Description	Units
Type	Flag for culture type: Pure Culture = 1 strain; Coculture = 2 strains.	unitless
Strain1	Strain name for pure culture type and first strain for coculture type	unitless
Strain2	Strain name for second strain for coculture type	unitless

Culture_ID	Unique ID for culture tube in format [AAAAAAA]-[BBBBBBB]-[C][DD]-[EEE]-Rep[F]-[G] where AAAAAAA = Strain1; BBBBBBB = Strain2 (Coculture type only); C = Light_Color (W or B); DD = Light_Intensity; EEE = N_source; F = Replicate; G = Transfer	unitless
N_source	Concentration and type of inorganic nitrogen provided as a nitrogen source	unitless
Light_Intensity	Intensity of light that the cultures were cultivated under	micromoles photones per square meter per second (umol photons m-2 s-1)
Light_Color	Spectra of light that the cultures were cultivated under (White or Blue)	unitless
Replicate	Biological replicate (A, B, or C)	unitless
Transfer	Transfer number: 0 for cultures followed over a single growth curve; 1 for first transfer of cultures followed over multiple growth curves; 2 for second transfer of culture followed over multiple growth curves	unitless
Time	Time (in days) of data collection measured from time of inoculation	days
Cell_Concentration_FCM	Concentration of total cyanobacteria cells as determined by flow cytometry	cells per milliliter (cells mL-1)
Cell_Concentration_QPCR_MIT0915	Concentration of Prochlorococcus MIT0915 cells as determined by quantitative PCR	cells per milliliter (cells mL-1)
Cell_Concentration_QPCR_MIT0917	Concentration of Prochlorococcus MIT0917 cells as determined by quantitative PCR	cells per milliliter (cells mL-1)
Cell_Concentration_QPCR_MIT1214	Concentration of Prochlorococcus MIT1214 cells as determined by quantitative PCR	cells per milliliter (cells mL-1)
Nitrite_Concentration	Extracellular concentration of dissolved nitrite in the culture; BDL = below detection limit of 1 micromole per liter (umol/L)	micromoles nitrate per liter (umol nitrite L-1)

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Guava easyCyte 12HT Flow Cytometer (MilliporeSigma, Burlington, MA, USA)
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)

Dataset-specific Instrument Name	Synergy 2 Plate Reader (BioTek Instruments, Winooski, VT, USA)
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.

Dataset-specific Instrument Name	CFX96 Thermocycler (Bio-Rad, Hercules, CA, USA)
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

[table of contents | back to top]

Project Information

Features and implications of nitrogen assimilation trait variability in populations of Prochlorococcus (Prochlorococcus N assimilation)

Coverage: Laboratory studies (MIT)

NSF Award Abstract:
The marine bacterium, *Prochlorococcus*, is a central part of the food web in the subtropical open ocean, one of the largest biomes on the planet. Like plants on land, *Prochlorococcus* and other phytoplankton are capable of photosynthesis, harnessing light to convert carbon dioxide into sugars and other organic matter. This matter feeds all the life in the sea. Akin to terrestrial plants, *Prochlorococcus* requires additional nutrients or "fertilizer" to grow and photosynthesize. Among these nutrients, nitrogen is often scarce across much of the sunlit ocean. Thus, understanding the means through which nitrogen is obtained and used by *Prochlorococcus* has important consequences for understanding how the nitrogen and carbon cycles are coupled in the ocean. Not all *Prochlorococcus* cells have the genetic capacity to use all sources of inorganic nitrogen, i.e. nitrate, nitrite, and ammonium. Some can use all three, some the last two, and some only ammonium. Cells that can use nitrate must sequentially transform it to nitrite and then to ammonium before they can make the building blocks for proteins. The genesis of this project derives from the observation that some *Prochlorococcus* cells release nitrite into the seawater during growth on nitrate. This project examines this feature of the physiology of these cell lines, and asks whether cells that release nitrite can support the growth of other cells than cannot use nitrate, in effect creating a cross-feeding situation that could make the system more robust. Understanding the drivers behind the coexistence of cells with different ways of obtaining nitrogen, a key currency in the ocean, will provide important insights on the flow of nitrogen in marine ecosystems. This project also sheds light on the structure of interactions between microbes and provides the broader scientific community (for instance, those studying diverse microbiomes related to human health and disease or agriculture) a new perspective on how microbes form beneficial partnerships. This project supports immersive laboratory-based research experiences for undergraduate students, who design and execute experiments directly related to the overall project goals. The project further supports the work of the investigators to engage with the general public on topics related to phytoplankton, photosynthesis, and the ecosystem services provided by these marine organisms.

In the low-light adapted LLI clade of *Prochlorococcus*, the focus of this project, nearly all cells possess the downstream half of the nitrate assimilation pathway (for the assimilation of nitrite). Only a fraction of LLI cells, however, have the complete nitrate assimilation pathway. Incomplete assimilatory nitrate reduction, with concomitant nitrite release, has been observed for LLI cells during growth on nitrate as the sole nitrogen source. Further, the nitrite released by cells growing on nitrate can support the growth of *Prochlorococcus* that can use nitrite but not the more oxidized nitrate. Overall, within a group of closely-related *Prochlorococcus*, there is genotypic and phenotypic diversity related to the production and consumption of nitrite, a central intermediate in the nitrogen cycle. The investigators propose to further develop *Prochlorococcus*as a model system to explore nitrite cycling within populations and provide new insights on how trait variability and the selection of complementary functions facilitates robustness and/or resiliency in microbial populations. The overarching hypothesis is that the population-level assembly of distinct functional types of *Prochlorococcus* emerges through interactions that are mediated, in part, by cross-feeding of nitrite. To address this broad hypothesis, the investigators are focusing on the following objectives: 1) assessing the physiological underpinnings of incomplete assimilatory nitrate reduction and nitrite release through in-vitro biochemical characterization of nitrite reductase enzymes and transcription profiling of cells subjected to light and temperature stress, 2) examining the nitrite production and consumption rates of *Prochlorococcus* strains across environmental gradients such as light, temperature, and nutrient availability in order to constrain the environmental parameters that modulate nitrite cycling, and 3) determining the frequencies and activities of nitrogen assimilation genotypes within laboratory and field populations, under varying environmental conditions and perturbations. Outcomes from objectives 1 and 2 help to constrain the tradeoffs associated with incomplete nitrate reduction and the release of nitrite (a valuable commodity to nitrogen limited cells) to facilitate modelling and interpretation of how partnerships between nitrogen assimilation genotypes are structured. These insights help to direct experiments in Objective 3, which aims to examine controlled laboratory co-cultures and field populations in order to produce quantitative data on the emergent features of *Prochlorococcus* populations where interactions are mediated by the cross-feeding of nitrite. These data are being used to develop an improved understanding of how interactions mediated by a common public good might give rise to emergent properties of populations, including resilience to perturbation and greater population-wide efficiency in nitrogen assimilation.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2048470

[table of contents | back to top]