Diatom metabolites under P-limited and P-replete growth from laboratory cultures in July of 2013

Website: https://www.bco-dmo.org/dataset/859671
Data Type: experimental
Version: 1
Version Date: 2021-08-27

Project
» Identifying and quantifying new markers of microbially mediated nutrient flow in the ocean (Microbial metabolites)

Program
» Marine Microbiology Initiative (MMI)

Contributors	Affiliation	Role
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Abstract
Diatom metabolites under P-limited and P-replete growth. Phosphorus limitation is pervasive in the oligotrophic surface ocean and marine microorganisms use different strategies to survive, and thrive, at these low nutrient levels. Eukaryotic algae such as diatoms are extremely sensitive to phosphorus limitation and recent transcriptomics work has suggested that multiple cellular processes are affected under these growth conditions. Metabolomics is the systematic study of intra- and extracellular metabolites, i.e. the end products of microbial metabolism. As such, metabolomics complements genomics and transcriptomics through the identification and quantification of metabolic intermediates that reflect cellular physiology. Here, we applied intracellular metabolomics to examine the differential response of the model diatom Thalassiosira pseudonana to phosphate-replete and phosphate-limited growth conditions. We focused on metabolites from the purine and pyrimidine biochemical pathways, due to their role in phosphorus cycling associated with nucleic acid synthesis. Under phosphate-replete conditions, T. pseudonana stored nucleotides with phosphate moieties such as adenosine 5'-monophosphate (AMP) and, to a lesser extent, inosine 5'-monophosphate (IMP). In contrast, under phosphate-limited conditions, T. pseudonana had higher concentrations of adenine, inosine, and adenosine all of which lack phosphate moieties. Furthermore, based on previously published transcriptomics data, T. pseudonana differentially regulates select genes that can alter these nucleic acid precursors through the gain or loss of the phosphate moiety. Thus, our analysis of the metabolomics and transcriptomics data converged upon the sensitivity of the purine biochemical pathway to phosphorus availability.

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Coverage

Temporal Extent: 2013-06

Dataset Description

Raw Spectral Data Files are available in the MetaboLights database under study ID "MTBLS154" (https://www.ebi.ac.uk/metabolights/MTBLS154/). Sample, assay, and metabolite information in ISAtab format are also available from that MetaboLights study.

Methods & Sampling

Metadata from "MTBLS154: Phosphorus availability regulates intracellular nucleotides in marine eukaryotic phytoplankton" at https://www.ebi.ac.uk/metabolights/MTBLS154/samples

Sample Collection:

Thalassiosira pseudonana (CCMP number 1335) was cultured axenically in a modified version of L1 media made with Turks Island Salts in order to reduce the background concentration of dissolved organic carbon in the media. The phosphate-replete treatment contained 36 µM PO4 and the phosphate-limited treatment had 0.4 µM PO4. Prior to the experiment, the culture had been maintained through two culture transfers of phosphate-replete or phosphate-limited media. The experiment began with the addition of 30 ml of T. pseudonana in exponential phase to two-thirds of the flasks which contained 300 ml of media. The remaining one-third of the flasks were designated as cell-free controls. Two flasks with cells and one cell-free control for each media (phosphate-replete or phosphate-limited) were sampled at four time points: 0, 2, 8, and 10 days.

Extraction:

The intracellular metabolites were extracted using a method modified from a protocol previously described in Rabinowitz and Kimball (2007). Briefly, the filter was extracted three times with ice-cold extraction solvent (acetonitrile:methanol:water with 0.1 M formic acid, 40:40:20). The combined extracts were neutralized with ammonium hydroxide and dried in a vacufuge.

The samples for the targeted mass spectrometry analysis were re-dissolved in 95:5 (v/v) water:acetonitrile and combined with deuterated biotin (final concentration 0.05 µg/ml)as an internal standard.

For untargeted analysis, the extracts had to undergo an additional de-salting step prior to analysis. Therefore, the dried extracts were re-dissolved in 0.01 M hydrochloric acid and extracted using a 50 mg/1 cc PPL cartridge following the protocol of Dittmar et al. (2008). The resulting methanol extracts were re-dissolved in 95:5 water:acetonitrile and deuterated biotin.

Chromatography:

For targeted metabolomics analysis, the samples were analyzed with a Synergi 4 µm Fusion – RP 80 Å, 150 × 2.00 mm column (Phenomenex, Torrance, CA) coupled to a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer. The chromatography gradient was: an initial hold of 95% A (0.1% formic acid in water) : 5% B (0.1% formic acid in acetonitrile) for 2 minutes, ramp to 65% B from 2 to 20 minutes, ramp to 100% B from 20 to 25 min, and hold until 32.5 minutes. The column was re-equilibrated for 7 min between samples with solvent A. Each metabolite was quantified using multiple reaction monitoring (MRM) mode with optimal parameters determined from infusion of authentic standards. Eight-point external calibration curves (0.5, 1, 5, 10, 50, 100, 250, and 500 ng/ml) were generated for each compound by plotting peak area against concentration.

For untargeted metabolomics analysis, LC separation was performed using a Synergi Fusion reversed phase column (Phenomenex, Torrance, CA) with the same gradient as for targeted analysis.

Mass spectroscopy:

For targeted analysis, samples were analyzed with a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer. This instrument allows polarity switching between positive and negative ion mode within a single LC run. Analysis of authentic standards was used to determine the optimal ionization mode for each metabolite.

For untargeted analysis, samples were analyzed in both negative and positive ion mode with liquid chromatography (LC) coupled by electrospray ionization to a 7-Tesla Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR MS). In parallel to the FT acquisition, four data dependent MS/MS scans

were collected at nominal mass resolution in the ion trap (LTQ). Samples were analyzed in random order with a pooled sampled run every six samples in order to assess instrument variability.

Metabolite identification:

The targeted metabolomics compound identifications were based on measurements of authentic standards on the same mass spectrometer. Note that the LC/mass spectrometry system cannot distinguish between leucine and isoleucine, therefore the data are presented as 'leucine/isoleucine'.

Data Processing Description

Targeted metabolomics data The resulting data were converted to mzML files using the msConvert tool (Chambers et al., 2012) and processed with MAVEN (Melamud et al., 2010). The concentration of the metabolites in the targeted mass spectrometry data is given in ng/ml. Note that the concentration of these metabolites in the cell-free controls has been subtracted from the corresponding treatment with Thalassiosira.

Untargeted metabolomics data Data were collected as XCalibur RAW files which were converted to mzXML files using the msConvert tool within ProteoWizard (Chambers et al., 2012). Features were extracted from the LC-MS data using XCMS (Smith et al., 2006), where a feature is defined as a unique combination of a mass-to-charge (m/z) ratio and a retention time. Peak finding was performed with the centWave algorithm (Tautenhahn et al., 2008), and only peaks that fit a Gaussian shape were retained. Features were aligned across samples based on retention time and m/z value using the group.nearest function in XCMS; fillPeaks was used to reconsider features missed in the initial peak finding steps. CAMERA was used 1) to find compounds differing by adduct ion and stable isotope composition (Kuhl et al., 2012) and 2) to extract the intensities and m/z values for the associated MS/MS spectra.

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Related Publications

Chambers, M. C., Maclean, B., Burke, R., Amodei, D., Ruderman, D. L., Neumann, S., … Mallick, P. (2012). A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology, 30(10), 918–920. doi:10.1038/nbt.2377
Software

Dittmar, T., Koch, B., Hertkorn, N., & Kattner, G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography: Methods, 6(6), 230–235. doi:10.4319/lom.2008.6.230
Methods

Kuhl, C., Tautenhahn, R., Böttcher, C., Larson, T. R., & Neumann, S. (2011). CAMERA: An Integrated Strategy for Compound Spectra Extraction and Annotation of Liquid Chromatography/Mass Spectrometry Data Sets. Analytical Chemistry, 84(1), 283–289. doi:10.1021/ac202450g
Methods

Kujawinski, E. B., Longnecker, K., Alexander, H., Dyhrman, S. T., Fiore, C. L., Haley, S. T., & Johnson, W. M. (2017). Phosphorus availability regulates intracellular nucleotides in marine eukaryotic phytoplankton. Limnology and Oceanography Letters, 2(4), 119–129. doi:10.1002/lol2.10043
Results

Longnecker, K., & Kujawinski, E. B. (2017). Mining mass spectrometry data: Using new computational tools to find novel organic compounds in complex environmental mixtures. Organic Geochemistry, 110, 92–99. doi:10.1016/j.orggeochem.2017.05.008
Results

Melamud, E., Vastag, L., & Rabinowitz, J. D. (2010). Metabolomic Analysis and Visualization Engine for LC−MS Data. Analytical Chemistry, 82(23), 9818–9826. doi:10.1021/ac1021166
Software

Rabinowitz, J. D., & Kimball, E. (2007). Acidic Acetonitrile for Cellular Metabolome Extraction fromEscherichia coli. Analytical Chemistry, 79(16), 6167–6173. doi:10.1021/ac070470c
Methods

Smith, C. A., Want, E. J., O'Maille, G., Abagyan, R., & Siuzdak, G. (2006). XCMS: Processing Mass Spectrometry Data for Metabolite Profiling Using Nonlinear Peak Alignment, Matching, and Identification. Analytical Chemistry, 78(3), 779–787. doi:10.1021/ac051437y
Software

Tautenhahn, R., Böttcher, C., & Neumann, S. (2008). Highly sensitive feature detection for high resolution LC/MS. BMC Bioinformatics, 9(1). doi:10.1186/1471-2105-9-504
Methods

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Related Datasets

IsRelatedTo

Longnecker, K., Kujawinski, E. (2015) MTBLS154: Phosphorus availability regulates intracellular nucleotides in marine eukaryotic phytoplankton. MetaboLights Database. Release Date: 2015-01-27. Available at https://www.ebi.ac.uk/metabolights/MTBLS154/

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	For targeted analysis, samples were analyzed with a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer. For untargeted analysis, samples were analyzed in both negative and positive ion mode with liquid chromatography (LC) coupled by electrospray ionization to a 7-Tesla Fourier-transform ion cyclotron resonance mass spectrometer (FT-ICR MS).
Generic Instrument Description	General term for instruments used to measure the mass-to-charge ratio of ions; generally used to find the composition of a sample by generating a mass spectrum representing the masses of sample components.

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Project Information

Identifying and quantifying new markers of microbially mediated nutrient flow in the ocean (Microbial metabolites)

Coverage: Laboratory cultures

In support of developing new protocols to detect the products of microbial metabolism in seawater to understand the influence of marine microbial communities and their activities on the chemical composition of their surroundings.

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Program Information

Marine Microbiology Initiative (MMI)

Website: https://www.moore.org/initiative-strategy-detail?initiativeId=marine-microbiology-initiative

A Gordon and Betty Moore Foundation Program.

Forging a new paradigm in marine microbial ecology:

Microbes in the ocean produce half of the oxygen on the planet and remove vast amounts of carbon dioxide, a greenhouse gas, from the atmosphere. Yet, we have known surprisingly little about these microscopic organisms. As we discover answers to some long-standing puzzles about the roles that marine microorganisms play in supporting the ocean's food webs and driving global elemental cycles, we realized that we still need to learn much more about what these organisms do and how they do it—including how they evolved and contribute to our ocean's health and productivity.

The Marine Microbiology Initiative seeks to gain a comprehensive understanding of marine microbial communities, including their diversity, functions and behaviors; their ecological roles; and their origins and evolution. Our focus has been to enable researchers to uncover the principles that govern the interactions among microbes and that govern microbially mediated nutrient flow in the sea. To address these opportunities, we support leaders in the field through investigator awards, multidisciplinary team research projects, and efforts to create resources of broad use to the research community. We also support development of new instrumentation, tools, technologies and genetic approaches.

Through the efforts of many scientists from around the world, the initiative has been catalyzing new science through advances in methods and technology, and to reduce interdisciplinary barriers slowing progress. With our support, researchers are quantifying nutrient pools in the ocean, deciphering the genetic and biochemical bases of microbial metabolism, and understanding how microbes interact with one another. The initiative has five grant portfolios:

Individual investigator awards for current and emerging leaders in the field.
Multidisciplinary projects that support collaboration across disciplines.
New instrumentation, tools and technology that enable scientists to ask new questions in ways previously not possible.
Community resource efforts that fund the creation and sharing of data and the development of tools, methods and infrastructure of widespread utility.
Projects that advance genetic tools to enable development of experimental model systems in marine microbial ecology.
We also bring together scientists to discuss timely subjects and to facilitate scientific exchange.

Our path to marine microbial ecology was a confluence of new technology that could accelerate science and an opportunity to support a field that was not well funded relative to potential impact. Around the time we began this work in 2004, the life sciences were entering a new era of DNA sequencing and genomics, expanding possibilities for scientific research – including the nascent field of marine microbial ecology. Through conversations with pioneers inside and outside the field, an opportunity was identified: to apply these new sequencing tools to advance knowledge of marine microbial communities and reveal how they support and influence ocean systems.

After many years of success, we will wind down this effort and close the initiative in 2021. We will have invested more than $250 million over 17 years to deepen understanding of the diversity, ecological activities and evolution of marine microbial communities. Thanks to the work of hundreds of scientists and others involved with the initiative, the goals have been achieved and the field has been profoundly enriched; it is now

positioned to address new scientific questions using innovative technologies and methods.

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Funding

Funding Source	Award
Gordon and Betty Moore Foundation: Marine Microbiology Initiative (MMI)	GBMF3304

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