Intracellular metabolites from an experimental manipulation of marine microorganisms from samples collected during R/V Knorr cruise KN210-04 in May of 2013

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Project
» The Metabolic Response of Coastal Bacteria to Mortality-Derived Phytoplankton Dissolved Organic Matter (MortalityDOM)

Contributors	Affiliation	Role
Kujawinski, Elizabeth	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
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Abstract
Intracellular metabolites from an experimental manipulation of marine microorganisms. Seawater used in the experiments was collected off the northeastern corner of South America at 9.75 N, 55.3 W from 70 m. The marine environment holds one of the largest pools of reduced organic carbon on Earth. Within this organic carbon are metabolites produced as a result of microbial activity. This project considers the effects of predation and viral lysis on the metabolites found in the surface ocean. Using seawater from the surface ocean, we experimentally manipulated levels of predation and viral lysis. At the beginning and end of the experiment, we sampled the intracellular and extracellular metabolites. The extracts were analyzed using liquid chromatography-based targeted and untargeted metabolomics methods that we have modified for use with marine samples. The untargeted metabolomics data revealed a complex mixture of organic compounds in all of the experimental treatments. However, changes in the majority of the features could not be linked to viral lysis or predation. Within the targeted metabolomics data, increased intracellular levels of osmolytes (glycine betaine, dimethylsulfoniopropionate, proline, and ectoine) were observed under conditions with limited grazing or viral lysis. The molecular insights derived here will explicitly inform our understanding of microbial processes in the surface ocean and the subsequent impacts on the global carbon cycle.

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Coverage

Spatial Extent: **Lat**:9.75 **Lon**:-55.3
Temporal Extent: 2021-05-05

Dataset Description

Raw Spectral Data Files are available in the MetaboLights database under study ID "MTBLS461" (https://www.ebi.ac.uk/metabolights/MTBLS461/). Sample, assay, and metabolite information in ISAtab format are also available from that MetaboLights study.

Methods & Sampling

Metadata from "MTBLS461: Intracellular metabolites from an experimental manipulation of marine microorganisms"

at https://www.ebi.ac.uk/metabolights/MTBLS461/samples

Sample Collection:

Seawater for the incubation experiments was collected using 10 l Niskin bottles attached to a CTD/rosette system. Seawater was collected off the northeastern corner of South America at 9.75 N, 55.3 W from 70 m on May 5th, 2013. Silicone tubing was used to collect water from the Niskins and the tubing was placed in the bottom of polycarbonate carboys in order to minimize turbulence during sample collection. The seawater was first filtered through a 0.2 µm Sterivex (Millipore) to obtain cell-free seawater. To obtain cell- and virus-free seawater, tangential flow filtration using a recirculating Prep/Scale tangential flow ultrafilter (Millipore) with a 30 kDa molecular mass cut-off was used.

5 different experimental treatments were established: 1) a whole seawater control, 2) 20% whole seawater with cell-free seawater, 3) 45% whole seawater with cell-free seawater, 4) 20% whole seawater with cell- and virus-free seawater, 5) 45% whole seawater with cell- and virus-free seawater. There were 3 x 2 l polycarbonate bottles established for each treatment. Each bottle held 2320 ml of fluid. 1 of the bottles was sampled immediately after the experiment was set up. The 2 remaining bottles were incubated for 1 day in an on-deck, flow-through incubator that allowed 10% of photosynthetically active radiation (PAR) to pass through its screening.

Extraction:

The intracellular metabolites were extracted using a method modified from a previously desbribed protocol (Rabinowitz & Kimball, 2007) . Briefly, the filter was extracted 3 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. The final extract volume was 100 µl.

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 a previously established protocol (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 4µ Fusion – RP 80A 150 x 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 min, ramp to 65% B from 2 to 20 min, ramp to 100% B from 20 to 25 min, and hold until 32.5 min. 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. 10-point external calibration curves (0.5, 1, 5, 10, 25, 50, 100, 250, 500, and 1000 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 negative 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, 4 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 6 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. All identifications were 'MSI Level 1' identifications based on the established criteria (Sumner et al., 2007).

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.

Untargeted metabolomics 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

Longnecker, K., & Kujawinski, E. B. (2020). Intracellular Metabolites in Marine Microorganisms during an Experiment Evaluating Microbial Mortality. Metabolites, 10(3), 105. doi:10.3390/metabo10030105
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

Sumner, L. W., Amberg, A., Barrett, D., Beale, M. H., Beger, R., Daykin, C. A., … Viant, M. R. (2007). Proposed minimum reporting standards for chemical analysis. Metabolomics, 3(3), 211–221. doi:10.1007/s11306-007-

0082-2
Methods

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. (2017) MTBLS461: Intracellular metabolites from an experimental manipulation of marine microorganisms. MetaboLights Database. Release Date: 2017-12-20. Available at https://www.ebi.ac.uk/metabolights/MTBLS461/

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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 metabolomics analysis, the samples were analyzed with a Synergi 4µ Fusion – RP 80A 150 x 2.00 mm column (Phenomenex, Torrance, CA) coupled to a Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer. 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.
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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Deployments

KN210-04

Website	https://www.bco-dmo.org/deployment/59057
Platform	R/V Knorr
Start Date	2013-03-25
End Date	2013-05-09
Description	Western Atlantic cruise started at Montevideo, Uruguay and ended at Bridgetown, Barbados. Science Objectives: 1. Characterize deep ocean dissolved organic matter in water masses of western Atlantic Ocean. 2. Characterize microbial community at selected stations and at selected depths. 3. Characterize metabolic capabilities of surface, mesopelagic and bathypelagic microbial consortia vis-a-vis the degradation of organic matter from each zone. 4. Examine metabolic and phylogenetic links between microbes in different marine zones (surface, meso-pelagic and bathypelagic depths). Science Activities: 1. Collection of discrete water samples by Niskin-bottles. 2. Collection of microbial communities from these water samples, by in-situ pumping, or by net-traps and net-tows. 3. Incubation experiments in lab and on deck. 4. Underway mass spectrometry and flow cytometry, from seawater intake. More information is available from the WHOI Cruise Planning Synopsis. Additional cruise information and original data are available from the NSF R2R Data Catalog.

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Project Information

The Metabolic Response of Coastal Bacteria to Mortality-Derived Phytoplankton Dissolved Organic Matter (MortalityDOM)

Coverage: Laboratory

NSF Award Abstract:
Microbes interact with one another through the exchange of chemicals dissolved in their surrounding waters. Decades of biochemical research have identified a small suite of chemicals that are required by microbes for growth and well-being. This limited suite is now being expanded with novel analytical tools based on mass spectrometry. In this project, the focus will be on chemicals that are released during the death of microbes, with particular attention paid to burst cells after viral infections and to the remnants of cells after grazing by protozoa (single celled organisms). These chemicals are not intentionally released by their producers, but they can still affect the growth and well-being of nearby bacteria and in turn the bacteria's ability to convert these molecules to carbon dioxide. The proposed comparison of the types and reactivities of chemicals released during the death of a brown tide alga will help improve models of carbon cycling in the coastal ocean. Two graduate students will be supported directly by this project. The proponent plans to teach two classes, one a mass spectrometry course, the other an environmental metabolomics course. It is anticipated that as part of the evolution of the metabolomics course, data-training for metabolomics would become part of the course.

Microbial consortia are exquisitely sensitive to chemical changes in their surroundings and the diversity of microbial communities evolves with the composition of available growth substrates and nutrients. Thus, interactions between microbes, through the milieu of dissolved organic matter (DOM), lie at the heart of the global carbon cycle and thus merit significant study and investigation. This project focuses on the molecules that are released during microbial mortality through viral lysis or protozoan grazing. Using novel mass spectrometry-based tools, this project links the composition of dissolved organic matter derived from microbial mortality with the ability of heterotrophic bacteria to remineralize these substrates. Metabolic parameters and carbon transformation rates will be determined as a function of DOM source to assess the impact of DOM type on microbial physiology and carbon turnover. Laboratory results from model organisms will be compared to field settings where the model organisms dominate planktonic communities. The project will generate a suite of molecules that can be used in future experiments as markers of microbial mortality and will provide quantitative comparisons between the reactivity of viral lysate and grazer-derived DOM. These results will support improved parameterizations of microbial networks and their impact on the global carbon cycle.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1634016

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