Metabolomic fingerprints of individual algal cells using the Single-Probe Mass Spectrometry technique from experiments conducted between May and August of 2017

Website: https://www.bco-dmo.org/dataset/746185
Data Type: experimental
Version: 1
Version Date: 2018-09-21

Project
» Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages (Creatine Cycling)

Contributors	Affiliation	Role
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Abstract
Single-probe Mass Spectrometry was used to obtain mass to charge ratio (m/z) and relative intensity values of marine dinoflagellate Scrippsiella trochoidea cells grown under different illumination levels and under nitrogen (N) limiting conditions.

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Coverage

Temporal Extent: 2017-05 - 2017-08

Dataset Description

These data were published in Sun et al., 2018.

A tabular version of this dataset is accessible by clicking the "Get Data" button on this page. The columns of the tabular dataset are described in the "Parameters" section on this page. This dataset is available as standard MS file format (.mzML) files within the following tar.gz file:
Algal_Cell_Data_mzML.tar.gz (2.4 GB, contains 20 .mzML files)

.mzML is a standard MS file format that can be viewed using freeware such as mMass (http://www.mmass.org/) and ProteoWizard (http://proteowizard.sourceforge.net/index.shtml).

Methods & Sampling

Methodology
The following are excerpts from Sun et al., 2018. Please refer to this publication for more details.

Non-axenic Scrippsiella trochoidea CCMP 3099 was originally obtained from the National Center for Marine Algae and Microbiota (Provasoil-Guillard NCMA, Boothbay Harbor, ME, United States). For maintenance, cultures were grown in L1 seawater media (Guillard and Ryther, 1962; Guillard et al., 1973; Guillard and Hargraves, 1993). Medium was prepared from natural seawater collected near Key West (salinity of 33), which was aged for at least 6 month in the dark and autoclaved. Maintenance and experimental cultures were grown in a light/dark incubator at 23–24°C and 30–40 µmol quanta·m-2 s-1 light under a 12-h light:12-h dark cycle.

For the light/dark comparisons, cultures were grown under replete conditions in full L1 media containing 880 µM NaNO3 and 36 µM NaH2PO4 (N/P ratio 24:1). Experimental cultures were started as a 1:10 inoculum from exponentially growing cultures into 1 L of media in 2.5 L Pyrex Fernbach flasks without shaking and monitored daily via cell counts and chlorophyll measurements. Cell counts were conducted by addition of 1% Lugol's iodine and direct counting of cells in 96-well microtiter plates using a dissection microscope. Dilutions were made as necessary and at least 10 wells containing 100 µL diluted culture were counted to average cell counts. Chlorophyll a was measured via fluorometry (Welschmeyer, 1994) by filtering 5 mL of culture in triplicate onto GF/F filters, over-night extraction with methanol, and quantification using a Turner Trilogy Laboratory Fluorometer. Cultures were grown into late-log phase (data not shown) and then sampled 3 h before and 3 h after the light was turned on. Cultures were sub-sampled for MS analysis, making sure to keep 'dark' sample exposure to light to a minimum by wrapping sampling tubes in aluminum foil.

N-deplete cultures were generated by first growing cells on L1 media with an N:P ratio of 2.4:1 (88 mM nitrate: 36 mM phosphate) analogous what has been previously described (Harke et al., 2017). This lower ratio stoichiometrically limited cultures in nitrogen and at least three transfers were performed to ensure no carryover from higher nutrient full L1 medium. Cultures were monitored daily via cell counts (see above), chlorophyll a quantification (see above), and quantification of nitrate/nitrite via a Vanadium reduction method (Miranda et al., 2001). Parallel cultures were set up in which one culture was allowed to run out of nitrogenous nutrients (N-deplete), while the culture (control) was fed additional nitrate every second day to bring total nitrate/nitrite concentrations back to starting levels. Once nitrate/nitrite levels dropped below the limit of detection (~1–2 µM) in the N-deplete culture, cultures were grown for an additional 24 h before sampling to ensure that N-depletion was complete. Both the N-deplete and replete cultures were then sampled for MS analysis of single cells.

Individual cells of S. trochoidea were analyzed via the 'Single-probe' MS techniques.
For the analysis, cells were deposited onto 0.2 µm polycarbonate membranes by gentle filtration, and the cells were rinsed with phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.47 mM KH2PO4; pH of 7.4) to remove culture medium. Filters were then placed on a home-built XYZ-translation stage system and spatial motion was controlled by a custom designed LabView software package (Lanekoff et al., 2012). The Single-probe tip (<10 µm) was then precisely insert into single S. trochoidea cells (typically ~20–30 µm cellular diameter) using a microscope as a guide. During the experiment, a syringe (250 µl; Hamilton, Co., Reno, NV, United States) was used to continuously provide the sampling solvent (acetonitrile; Sigma-Aldrich, St. Louis, MO, United States), and a liquid junction formed at the Single-probe tip to perform highly efficient extraction of cellular contents. The analytes were withdrawn by capillary action toward the nano-ESI emitter, and ionized for analysis using a Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States). Mass analyze parameters were as follows: mass resolution 60,000, +4 kV ionization voltage at positive ion mode (0.05–0.07 µA of ion current), 1 microscan, 100 ms max injection time, and automatic gain control on.

Data processing:
The Thermo Xcalibur Qual Browser (Thermo Scientific, Waltham, MA, United States) was used to export MS data (m/z values with relative intensities) as tab-delimited data files. As a conservative approach, only relatively abundant peaks with ion intensities > 103 were exported. This approach excluded 6% of low signal peaks as background while retaining 94% of total signal intensity. The relative ion intensities were normalized to the total ion current to minimize the influence induced by fluctuations of ion signals during experiments. The Geena 2 online software tool1 was then used for peak alignment (Romano et al., 2016), and the aligned m/z values were then used for comparisons. Parameters used in Geena 2 include analysis range (from 100 to 1500 m/z), maximum number of isotopic replicas (5), maximum delta between isotopic peaks (0.05 Da), and maximum delta for aligning replicates (0.01 Da). Metaboanalyst 3.02 was used to conduct statistical data analysis, including PLS-DA (partial least squares discriminant analysis) and t-tests (Xia et al., 2015; Xia and Wishart, 2016). PLS-DA was used to visualize differences in chemical composition profiles among treatment groups, while t-tests were applied to extract molecular peaks with significant abundance changes ($p < 0.05$). Finally, the online database METLIN3 was used to tentatively label all ions of interest (Smith et al., 2005; Guijas et al.,

2018), to perform hierarchical clustering, and to generate heat maps. Lastly, Pathos4 was used to attempt identification of significantly regulated metabolic pathways by considering all KEGG maps in all organisms (Leader et al., 2011).

Single-Probe description:
Detailed fabrication protocols of the Single-probe have been previously described (Pan et al., 2014; Rao et al., 2015; Sun et al., 2017). Briefly, a Single-probe has three components: a dual-bore quartz tubing [outer diameter (OD) 500 µm; inner diameter (ID) 127 µm, Friedrich & Dimmock, Inc., Millville, NJ, United States] pulled using a laser pipette puller (P-2000 micropipette puller, Sutter Instrument, Novato, CA, United States), a fused silica capillary (OD 105 µm; ID 40 µm, Polymicro Technologies, Phoenix, AZ, United States), and a nano-ESI emitter made from the same type of fused silica capillary. A Single-probe is fabricated by embedding a fused silica capillary and a nano-ESI emitter into both of the channels of the laser-pulled dual-bore quartz needle.

Data Processing Description

BCO-DMO Data Manager Processing Notes:
* added a conventional header with dataset name, PI name, version date
* modified parameter names to conform with BCO-DMO naming conventions

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Data Files

File
algae_cell_data.csv (Comma Separated Values (.csv), 20.92 MB) MD5:08a1374d7d0dc85ab2d14a2e34fbeaed Primary data file for dataset ID 746185

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Related Publications

Guijas, C., Montenegro-Burke, J. R., Domingo-Almenara, X., Palermo, A., Warth, B., Hermann, G., … Siuzdak, G. (2018). METLIN: A Technology Platform for Identifying Knowns and Unknowns. Analytical Chemistry, 90(5), 3156–3164. doi:10.1021/acs.analchem.7b04424
Methods

Guillard, R. R. L., & Hargraves, P. E. (1993). Stichochrysis immobilis is a diatom, not a chrysophyte. Phycologia, 32(3), 234–236. doi:10.2216/i0031-8884-32-3-234.1
Methods

Guillard, R. R. L., & Ryther, J. H. (1962). STUDIES OF MARINE PLANKTONIC DIATOMS: I. CYCLOTELLA NANA HUSTEDT, AND DETONULA CONFERVACEA (CLEVE) GRAN. Canadian Journal of Microbiology, 8(2), 229–239. doi:10.1139/m62-029
Methods

Guillard, R. R. L., Kilham, P., & Jackson, T. A. (1973). KINETICS OF SILICON-LIMITED GROWTH IN THE MARINE DIATOM THALASSIOSIRA PSEUDONANA HASLE AND HEIMDAL (= CYCLOTELLA NANA HUSTEDT)2. Journal of Phycology, 9(3), 233–237. doi:10.1111/j.1529-8817.1973.tb04086.x
Methods

Harke, M. J., Juhl, A. R., Haley, S. T., Alexander, H., & Dyhrman, S. T. (2017). Conserved Transcriptional Responses to Nutrient Stress in Bloom-Forming Algae. Frontiers in Microbiology, 8. doi:10.3389/fmicb.2017.01279
Methods

Lanekoff, I., Heath, B. S., Liyu, A., Thomas, M., Carson, J. P., & Laskin, J. (2012). Automated Platform for High-Resolution Tissue Imaging Using Nanospray Desorption Electrospray Ionization Mass Spectrometry. Analytical Chemistry, 84(19), 8351–8356. doi:10.1021/ac301909a

Methods

Leader, D. P., Burgess, K., Creek, D., & Barrett, M. P. (2011). Pathos: A web facility that uses metabolic maps to display experimental changes in metabolites identified by mass spectrometry. Rapid Communications in Mass Spectrometry, 25(22), 3422–3426. doi:10.1002/rcm.5245
Methods

Miranda, K. M., Espey, M. G., & Wink, D. A. (2001). A Rapid, Simple Spectrophotometric Method for Simultaneous Detection of Nitrate and Nitrite. Nitric Oxide, 5(1), 62–71. doi:10.1006/niox.2000.0319
Methods

Pan, N., Rao, W., Kothapalli, N. R., Liu, R., Burgett, A. W. G., & Yang, Z. (2014). The Single-Probe: A Miniaturized Multifunctional Device for Single Cell Mass Spectrometry Analysis. Analytical Chemistry, 86(19), 9376–9380. doi:10.1021/ac5029038
Methods

Pan, N., Rao, W., Standke, S. J., & Yang, Z. (2016). Using Dicationic Ion-Pairing Compounds To Enhance the Single Cell Mass Spectrometry Analysis Using the Single-Probe: A Microscale Sampling and Ionization Device. Analytical Chemistry, 88(13), 6812–6819. doi:10.1021/acs.analchem.6b01284
Methods

Rao, W., Pan, N., & Yang, Z. (2016). Applications of the Single-probe: Mass Spectrometry Imaging and Single Cell Analysis under Ambient Conditions. Journal of Visualized Experiments, (112). doi:10.3791/53911
Methods

Smith, C. A., Maille, G. O., Want, E. J., Qin, C., Trauger, S. A., Brandon, T. R., … Siuzdak, G. (2005). METLIN. Therapeutic Drug Monitoring, 27(6), 747–751. doi:10.1097/01.ftd.0000179845.53213.39
Methods

Sun, M., Tian, X., & Yang, Z. (2017). Microscale Mass Spectrometry Analysis of Extracellular Metabolites in Live Multicellular Tumor Spheroids. Analytical Chemistry, 89(17), 9069–9076. doi:10.1021/acs.analchem.7b01746
Methods

Sun, M., Yang, Z., & Wawrik, B. (2018). Metabolomic Fingerprints of Individual Algal Cells Using the Single-Probe Mass Spectrometry Technique. Frontiers in Plant Science, 9. doi:10.3389/fpls.2018.00571
Results

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments. Limnology and Oceanography, 39(8), 1985–1992. doi:10.4319/lo.1994.39.8.1985
Methods

Xia, J., & Wishart, D. S. (2016). Using MetaboAnalyst 3.0 for Comprehensive Metabolomics Data Analysis. Current Protocols in Bioinformatics, 55(1), 14.10.1–14.10.91. doi:10.1002/cpbi.11
Methods

Xia, J., Sinelnikov, I. V., Han, B., & Wishart, D. S. (2015). MetaboAnalyst 3.0—making metabolomics more meaningful. Nucleic Acids Research, 43(W1), W251–W257. doi:10.1093/nar/gkv380
Methods

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Related Datasets

Different Version

Mei, S., Yang, Z., & Wawrik, B. (2018). Mass Spectra For Single Scrippsiella Trochoidea Cells: Light Vs. Dark & Replete Vs. N Limiting Conditions (Version 1) [Data set]. Zenodo. https://doi.org/10.5281/zenodo.1188486

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Parameters

Parameter	Description	Units

light1_cell1_mz	Mass to charge ratio (m/z) of light contition experiment replicate 1 cell 1	dimensionless
light1_cell1_intensity	Relative intensity of light contition experiment replicate 1 cell 1	unitless
light1_cell2_mz	Mass to charge ratio (m/z) of light contition experiment replicate 1 cell 2	dimensionless
light1_cell2_intensity	Relative intensity of light contition experiment replicate 1 cell 2	unitless
light1_cell3_mz	Mass to charge ratio (m/z) of light contition experiment replicate 1 cell 3	dimensionless
light1_cell3_intensity	Relative intensity of light contition experiment replicate 1 cell 3	unitless
light1_cell4_mz	Mass to charge ratio (m/z) of light contition experiment replicate 1 cell 4	dimensionless
light1_cell4_intensity	Relative intensity of light contition experiment replicate 1 cell 4	unitless
light1_cell5_mz	Mass to charge ratio (m/z) of light contition experiment replicate 1 cell 5	dimensionless
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light1_cell6_mz	Mass to charge ratio (m/z) of light contition experiment replicate 1 cell 6	dimensionless
light1_cell6_intensity	Relative intensity of light contition experiment replicate 1 cell 6	unitless
light2_cell1_mz	Mass to charge ratio (m/z) of light contition experiment replicate 2 cell 1	dimensionless
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light2_cell2_mz	Mass to charge ratio (m/z) of light contition experiment replicate 2 cell 2	dimensionless
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replete5_cell5_mz	Mass to charge ratio (m/z) of replete contition experiment replicate5 cell5	dimensionless
replete5_cell5_intensity	Relative intensity of replete contition experiment replicate5 cell5	unitless
replete5_cell6_mz	Mass to charge ratio (m/z) of replte contition experiment replicate6 cell6	dimensionless
replete5_cell6_intensity	Relative intensity of replete contition experiment replicate6 cell6	unitless

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Instruments

Dataset-specific Instrument Name	Thermo LTQ Orbitrap XL mass spectrometer
Generic Instrument Name	Mass Spectrometer
Dataset-specific Description	Thermo LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Waltham, MA, United States). Mass analyze parameters were as follows: mass resolution 60,000, +4 kV ionization voltage at positive ion mode (0.05–0.07 µA of ion current), 1 microscan, 100 ms max injection time, and automatic gain control on.
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

Collaborative Research: Creatine Cycling in Marine Bacterial and Phytoplankton Assemblages (Creatine Cycling)

Coverage: Atlantic bight

NSF Award Abstract:
High rates of dissolved organic nitrogen (DON) production and utilization in aquatic systems are typically attributed to microbial activity. Though it is known that there is a tight coupling between the production and consumption of biologically available DON, the composition, dynamics, and ecological significance of this rapidly cycled DON pool are less well understood. This proposal focuses on a component of the DON pool, creatine,

which is historically understood as a product of metazoan activity, but appears to be both produced by phytoplankton and consumed by marine bacteria. Creatine is present in seawater in measurable quantities, which led to the hypothesis that creatine may be a significant component of the marine DON cycle. DON cycling likely has a bearing on fundamental marine ecosystem processes with large implications for carbon and nitrogen turnover on a global scale. Broader impacts of this project will include outreach that focuses on connecting scientists with K-12 students through research experiences for teachers and lesson development in collaboration with the K20 Center for Educational and Community Renewal, a statewide education research and development center at the University of Oklahoma. The project will integrate the research with inquiry-based teaching of rural secondary science teachers through Authentic Research Experiences in oceanographic science and microbial ecology. The K20 network includes 96% of Oklahoma schools, providing a unique opportunity to impact STEM education in Oklahoma.

The results of this project will help develop a better understanding of DON cycling, the ecological context of creatine uptake activity, and identify both creatine-producing and consuming organisms in the marine environment. The importance of creatine cycling will be assessed via 15N tracer studies along the natural coastal-to-offshore productivity gradient observed in the North Atlantic. Tracer and molecular approaches will be used to investigate the importance of phytoplankton vs. bacteria in creatine uptake and, the taxonomic identities of creatine-utilizing bacteria will be interrogated via molecular, stable isotope probing (SIP), and RT-qPCR approaches.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1634630

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