

# Sample and treatment information from mass spectrometry assays of metabolite experiments on *Thalassiosira pseudonana* 1335 inoculated with three bacterial strains

**Website:** <https://www.bco-dmo.org/dataset/826670>

**Data Type:** experimental

**Version:** 1

**Version Date:** 2020-10-14

## Project

» [Metabolic Currencies of the Ocean Carbon Cycle](#) (Metabolic Currencies)

» [Assessing the Movement of Carbon Currencies Between Marine Microbes](#) (Carbon Currencies Microbes)

## Program

» [Marine Microbiology Initiative](#) (MMI)

Contributors	Affiliation	Role
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## Abstract

This dataset includes information about samples and treatments analyzed with mass spectrometry. Bacteria *Ruegeria pomeroyi* DSS-3, *Stenotrophomonas* sp. SKA14, and *Polaribacter dokdonensis* MED152 were collected 8, 24, and 49 hours after individual inoculation into a diatom *Thalassiosira pseudonana* culture and resulting metabolites were analyzed. Raw data files are located at Metabolights, MetaboLights accession number MTBLS1751.

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## Coverage

**Temporal Extent:** 2018 - 2018

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## Dataset Description

The study examined the ability of bacteria to draw down exometabolites from the culture medium as detected by novel approaches in mass spectrometry (MS). See related dataset on parallel experiments using nuclear magnetic resonance (NMR) analysis. This dataset includes information about samples and treatments analyzed with MS. Bacteria *Ruegeria pomeroyi* DSS-3, *Stenotrophomonas* sp. SKA14, and *Polaribacter dokdonensis* MED152 were collected 8, 24, and 49 hours after individual inoculation into a diatom *Thalassiosira pseudonana* culture and resulting metabolites were analyzed.

Raw data files are located at Metabolights, MetaboLights accession number MTBLS1751:

<https://www.ebi.ac.uk/metabolights/MTBLS1751/files>

<https://www.ebi.ac.uk/metabolights/MTBLS1751/samples>

## Methods & Sampling

An axenic *Thalassiosira pseudonana* 1335 culture (National Center for Marine Algae) was grown in 500 ml of L1 +Si medium[1] in a 600 ml vented tissue culture flask at 18 °C under 16 h light at 160  $\mu\text{mol photons/m}^2/\text{s}$  and 8 h dark. After the diatom cultures had been growing for 7 d, bacteria pre-grown in 1/2 YTSS medium at 30 °C (*Ruegeria pomeroyi* DSS-3 (treatment A) and *Stenotrophomonas* sp. SKA14 (treatment D)) or 1/10 YTSS medium at 25 °C (*Polaribacter dokdonensis* (treatment E)) were washed 5 times in sterile L1 medium and inoculated into the diatom cultures and glucose controls at  $\sim 10^6$  (*Stenotrophomonas* sp. and *P. dokdonensis*) or  $\sim 10^7$  (*R. pomeroyi*) cells/ml. 3 or 4 replicates were collected for each co-culture. Axenic *T. pseudonana* and unamended L1+Si medium were maintained and sampled alongside the co-cultures as controls (treatment X and C). Samples were collected after 48 h.

Microbial cells were removed from co-cultures by filtration through 0.2  $\mu\text{m}$  pore-size filters at 8 h, 24 h, and 49 h after bacterial inoculation. Uninoculated medium served as the blank. Metabolites were derivatized with benzoyl chloride (Widner et al. in prep), extracted using a solid phase resin (Agilent, Bond Elut PPL), and analyzed using ultra high performance liquid chromatography coupled with electrospray ionization and tandem mass spectrometry (UHPLC-ESI-MSMS).

Data were processed using Skyline (Pino et al. 2017, Henderson et al. 2018) and MATLAB.

## Data Processing Description

### BCO-DMO Processing Notes:

- data submitted in Excel file "MTBLS1751 for BCO-DMO.xlsx" sheet "Sheet1" extracted to csv
- added 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
<b>diatom_matrix_mass-spec.csv</b> (Comma Separated Values (.csv), 3.24 KB) MD5:8cb2ada9cba70b0cd3b49b71defaae3d
Primary data file for dataset ID 826670

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## Related Publications

Ferrer-González, F. X., Widner, B., Holderman, N. R., Glushka, J., Edison, A. S., Kujawinski, E. B., & Moran, M. A. (2020). Resource partitioning of phytoplankton metabolites that support bacterial heterotrophy. *The ISME Journal*. doi:[10.1038/s41396-020-00811-y](https://doi.org/10.1038/s41396-020-00811-y)  
*Results*

Henderson, C. M., Shulman, N. J., MacLean, B., MacCoss, M. J., & Hoofnagle, A. N. (2018). Skyline Performs as Well as Vendor Software in the Quantitative Analysis of Serum 25-Hydroxy Vitamin D and Vitamin D Binding Globulin. *Clinical Chemistry*, 64(2), 408–410. doi:[10.1373/clinchem.2017.282293](https://doi.org/10.1373/clinchem.2017.282293)  
*Methods*

Pino, L. K., Searle, B. C., Bollinger, J. G., Nunn, B., MacLean, B., & MacCoss, M. J. (2020). The Skyline ecosystem: Informatics for quantitative mass spectrometry proteomics. *Mass Spectrometry Reviews*, 39(3),

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## Related Datasets

### IsSupplementTo

Frank Xavier Ferrer-González, Brittany Widner, Nicole Holderman, John Glushka, Arthur S Edison, Elizabeth B Kujawinski, Mary Ann Moran (2020) MTBLS1751: Resource Partitioning of Diatom Metabolites that Support Bacterial Heterotrophy in the Ocean (UPLC-MS assay) In: MetaboLights [Internet], EMBL-EBI, Wellcome Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK. Available from: <https://www.ebi.ac.uk/metabolights/MTBLS1751/samples>, MetaboLights study: MTBLS1751.

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## Parameters

Parameter	Description	Units
Sample_Name	sample name	unitless
Organism	identification of organism sampled	unitless
Organism_part	description of the part of the organism sampled	unitless
Treatment	treatment type	unitless
Time_elapsed_hr	time of sample collection	hours
Replicate	replicate number	unitless
MetaboLights_accession_number	accession number for data access at Metabolights	unitless

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## Instruments

<b>Dataset-specific Instrument Name</b>	Vanquish UHPLC system (Thermo Fisher Scientific)
<b>Generic Instrument Name</b>	High-Performance Liquid Chromatograph
<b>Dataset-specific Description</b>	Mass spectrometry samples were analyzed on a Vanquish UHPLC system (Thermo Fisher Scientific) coupled via heated electrospray ionization (H-ESI) to an ultrahigh resolution tribrid mass spectrometer, the Orbitrap Fusion Lumos (Thermo Fisher Scientific).
<b>Generic Instrument Description</b>	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.

<b>Dataset-specific Instrument Name</b>	Orbitrap Fusion Lumos (Thermo Fisher Scientific)
<b>Generic Instrument Name</b>	Mass Spectrometer
<b>Dataset-specific Description</b>	Mass spectrometry samples were analyzed on a Vanquish UHPLC system (Thermo Fisher Scientific) coupled via heated electrospray ionization (H-ESI) to an ultrahigh resolution tribrid mass spectrometer, the Orbitrap Fusion Lumos (Thermo Fisher Scientific).
<b>Generic Instrument Description</b>	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

### Metabolic Currencies of the Ocean Carbon Cycle (Metabolic Currencies)

#### *NSF Award Abstract:*

The roles of microbes in cycling carbon and nutrients in the ocean - the largest biological system on Earth - were initially described about 40 years ago. Now, it is known that half of Earth's primary production is carried out by marine phytoplankton, and half of that is recycled within weeks by marine bacteria. This proposal is a collaboration between microbiologists and chemists to identify the specific compounds that pass between phytoplankton and bacteria in surface ocean waters. Identifying the key chemicals of the ocean's microbial food web will provide insights into how the marine carbon cycle is regulated, generate data to improve ocean carbon models, and train new scientists at the interface of microbiology and chemistry. Hands-on learning opportunities in microbial ecology will be provided for high school students, both in the classroom and in marine ecosystems of the Georgia coast.

Phytoplankton metabolites that sustain the flow of carbon between microbial autotrophs and heterotrophs in the surface ocean carbon cycle will be identified in this project. A matrix of model systems consisting of bacteria-phytoplankton co-cultures will be used as biological assays for key metabolites based on expression patterns of bacterial transporter genes. The chemical identity of candidate metabolites and evaluation of their potential ecological role will be carried out by exometabolomic analysis of co-cultures with bacterial transporter mutants. Both advanced mass spectrometry and NMR will be used for metabolomics analysis, taking

advantage of the sensitivity and compound identification strengths of each. The distribution of candidate metabolites in the ocean microbiome and other microbial systems will be characterized by mining environmental sequence datasets for orthologous transporter genes. This project represents a novel approach to identifying metabolites important in microbiome function, compounds often difficult to address with standard chemical approaches because of their low concentrations and high biological demand.

## **Assessing the Movement of Carbon Currencies Between Marine Microbes (Carbon Currencies Microbes)**

**Coverage:** Laboratory cultures

In support of using advanced analytical instrumentation and protein characterization tools to measure the flux of nutrients exchanged between marine microbes.

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## **Program Information**

### **Marine Microbiology Initiative (MMI)**

**Website:** <https://www.moore.org/initiative-strategy-detail?initiativeld=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
<a href="#">NSF Division of Integrative Organismal Systems (NSF IOS)</a>	<a href="#">IOS-1656311</a>
<a href="#">Gordon and Betty Moore Foundation: Marine Microbiology Initiative (MMI)</a>	<a href="#">GBMF5503</a>

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