Thalassiosira pseudonana CCMP1335 endometabolite uptake by Ruegeria pomeroyi DSS-3

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Project
» Effects of Climate Change Variables on Microbial Autotroph-Heterotroph Carbon Flux (CC_Auto_Hetero_Fluxes)

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Abstract
The ocean's temperature increase has fundamental implications for physiological rates and processes of marine microbes. In this study, a marine diatom Thalassiosira pseudonana CCMP1335 was acclimated for three months at temperatures below (14°C), equal to (20°C), and above (28°C). Heterotrophic bacterium Ruegeria pomeroyi DSS-3 was inoculated into cultures, and transporter expression was compared between temperatures. R. pomeroyi transporter expression leveraged as a biosensor of available diatom exometabolites indicated temperature-related substitution of diatom osmolytes dimethylsulfoniopropionate (DMSP), dihydroxypropanesulfonate (DHPS), and homarine (dominating carbon transfer at lower temperatures) with glycine betaine and choline (dominating at higher temperatures). T. pseudonana endometabolome pools and biosynthetic pathway expression indicated increased availability of amino acids and glycerol-3-phosphate at higher temperatures. Overall trends across datasets supported a greater importance of organic sulfur compounds in diatom-bacterial metabolite transfer at lower temperatures and greater importance of organic nitrogen compounds at higher temperatures.

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Coverage

Location: University of Georgia, Athens GA 30602

Dataset Description

All the raw spectra, NMRPipe scripts for spectrum processing, and MATLAB scripts for data analysis are deposited in Metabolomics Workbench under Project ID PR001837 (https://www.metabolomicsworkbench.org/data/DRCCMetadata.php?Mode=Project...) (see related datasets).

Methods & Sampling

Temperature experiment: Axenic cultures of the diatom Thalassiosira pseudonana CCMP1335 (National Center for Marine Algae) were acclimated to three temperature conditions (below optimal: 14°C, optimal: 20°C, and above optimal: 28°C) for three months with weekly transfers (approximately 120 generations) in L1 medium with 35 ppt artificial seawater under 120 µmol photons m-2 s-1 (ULM-500 Light Meter, Walz) and a 16:8 h light:dark cycle. Diatom cultures were stepwise limited by B12 through three transfers. Ruegeria pomeroyi DSS-3 was grown overnight in ½ YTSS medium, harvested in exponential growth phase, washed five times in sterile artificial seawater at 6,000 RCF, and inoculated into diatom flasks with 4 replicates per acclimation temperature. Flasks were harvested at late exponential growth phase on days 3 (28°C), 4 (20°C), and 6 (14°C), with sampling occurring 7 h into the light cycle.

From axenic and inoculated flasks, cultures were sequentially filtered through 2.0 µm-pore-size Isopore filters (Millipore, Burlington, MA) and 0.2 µm-pore-size Supor filters (PALL, Port Washington, NY) to collect 600 ml for diatom endometabolits analysis, bacterial cells for RNA extraction. RNA filters were immediately flash-frozen in liquid nitrogen and stored at -80°C until processing. RNA was subsequently extracted from filters using the ZymoBIOMICS RNA Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol.

Stranded RNASeq libraries were prepared by the Joint Genome Institute (JGI) and sequenced on an Illumina NovaSeq.

Metabolites were analyzed by NMR spectroscopy using a 600 MHz AVANCE III HD instrument (Bruker) equipped with a 5 mm TCI cryoprobe and pulse programs of 1H-13C heteronuclear single quantum correlation (HSQC, hsqcetgpprsisp2.2 by Bruker nomenclature) and 1H-13C HSQC-total correlation spectroscopy (HSQC-TOCSY, hsqcdietgpsisp.2). TopSpin (Bruker) version 3.5 was used for NMR operation. Data were processed by NMRPipe.

Peak intensity was extracted by rNMR version 1.11 and data were analyzed by MATLAB (MathWorks) version R2023b. Peak intensity was normalized by biovolume and auto-scaled. Metabolites were annotated based on chemical shift (HSQC) and correlation information (HSQC-TOCSY). Chemical shift values for candidate peaks were obtained from the Biological Magnetic Resonance Data Bank and the Human Metabolome Database, and raw reference spectra from BMRB were used for validation.

Draw down experiment: Thalassiosira pseudonana CCMP1335 cells harvested from axenic flasks were rinsed from thawed filters into 10 ml sterile L1 medium, sonicated to lyse cells, and passed through a pre-combusted GF/F filter. Ruegeria pomeroyi DSS-3 was inoculated into a 2.7 ml aliquot of concentrated endometabolomes and grown for 10 h in 96-well plates with shaking at 30°C in dark conditions. Subsamples of 1 ml were collected at inoculation and at the end of the experiment for metabolite quantification. From these subsamples, 540 µl were mixed with 60 µL of the phosphate buffer and transferred to NMR tubes. Metabolites were analyzed by NMR spectroscopy using a 600 MHz AVANCE III HD instrument (Bruker) equipped with a 5 mm TCI cryoprobe and pulse programs of 1H-13C heteronuclear single quantum correlation (HSQC, hsqcetgpprsisp2.2 by Bruker nomenclature) and 1H-13C HSQC-total correlation spectroscopy (HSQC-TOCSY, hsqcdietgpsisp.2). TopSpin (Bruker) version 3.6.4 was used for NMR operation.

Data were processed by NMRPipe. Peak intensity was extracted by rNMR version 1.11 and data were analyzed by MATLAB (MathWorks) version R2023b. Peak intensity was normalized by biovolume and auto-scaled. Metabolites were annotated based on chemical shift (HSQC) and correlation information (HSQC-TOCSY). Chemical shift values for candidate peaks were obtained from the Biological Magnetic Resonance Data Bank and the Human Metabolome Database, and raw reference spectra from BMRB were used for validation.

Data Processing Description

Reads were filtered and trimmed using the JGI QC pipeline, followed by evaluation of artifact sequences by kmer matching (kmer=25) using BBDuk, allowing one mismatch; detected artifacts were trimmed from the 3 ´end of the reads. Quality trimming was performed using the phred trimming method set at Q6 and reads under the minimum length of 25 bases or 1/3 of the original read length were removed. Filtered reads from each library were aligned to the reference genome using HISAT2 version 2.2.0 and strand-specific coverage bigwig files were generated using deepTools v3.1. FeatureCounts was used to generate the raw gene counts, and DESeq2 (version 1.28.1) was used to determine relative expression differences between pairs of conditions. *T. pseudonana* gene annotations and biosynthesis pathways were based on Biocyc and PhyloDB. *R. pomeroyi* annotations were based on Roseobase (http://roseobase.org)

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Related Publications

Malin Olofsson, Mario Uchimiy, Frank X. Ferrer-González, Jeremy E. Schreier, McKenzie A. Powers, Christa B. Smith, Arthur S. Edison, and Mary Ann Moran. Metabolite Profiling of Temperature-Acclimated Marine Diatoms Co-cultured with Heterotrophic Bacteria. In Revision.
Results

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Related Datasets

IsRelatedTo

Moran, M.A., Hopkinson, B. Olofsson, M., McKenzie, P., Uchimiya, M. Temperature Effects on Metabolite-Mediated Autotroph-Heterotroph Carbon Transfer. Joint Genome Institution. https://doi.org/10.46936/10.25585/60001361

This data is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) website, the Metabolomics Workbench, https://www.metabolomicsworkbench.org where it has been assigned Project ID PR001837. The data can be accessed directly via it's Project doi: 10.21228/M88B0T. This work is supported by Metabolomics Workbench/National Metabolomics Data Repository (NMDR) (grant# U2C-DK119886), Common Fund Data Ecosystem (CFDE) (grant# 3OT2OD030544) and Metabolomics Consortium Coordinating Center (M3C) (grant# 1U2C-DK119889).. https://doi.org/doi:10.21228/M88B0T

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Parameters

Parameters for this dataset have not yet been identified

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Project Information

Effects of Climate Change Variables on Microbial Autotroph-Heterotroph Carbon Flux (CC_Auto_Hetero_Fluxes)

Coverage: Laboratory in Athens, GA, USA, and waters around Sapelo Island, GA, USA

NSF Award Abstract:
Phytoplankton in the surface ocean are responsible for roughly half of all photosynthesis on the planet. Much of the organic material created by these photosynthetic organisms is ultimately consumed by diverse marine bacteria with differing preferences for specific types of chemical compounds. This project investigates how climate change (temperature and CO2) might alter the types and amounts of organic compounds produced by different species of marine phytoplankton and the types and amounts of compounds transferred from phytoplankton to marine bacteria. Shifts in organic compounds transferred to bacteria could alter the distribution of bacterial species in the ocean, their growth rates and efficiencies, and flows of energy through the global ocean. This project helps scientists better understand the effects of climate change on marine ecosystems. Two graduate students and a postdoctoral researcher are supported by the project, receiving interdisciplinary training in biology, chemistry, and ocean sciences. Summer research internships in the PIs' laboratories are offered to AP Biology students enrolled at Cedar Shoals High School in Athens, GA, a school that serves a diverse social and economic community.

Much of the bacterial secondary production in the surface ocean is supported by rapid uptake of labile

metabolites released from phytoplankton, either directly through excretion and diffusion or indirectly through lysis and predation. This project investigates the effects of two climate change variables (temperature and CO2) on the metabolite pools produced and released by three model phytoplankton species (a diatom, a coccolithophore, and a cyanobacterium) and assesses changes in the composition and fate of metabolites transferred to bacteria. Phytoplankton species are being grown axenically at two different temperatures and CO2 concentrations in a factorial design and endo- and exometabolite composition is determined using NMR. A suite of phytoplankton physiological characteristics is measured and evaluated in the context of metabolite composition. Experiments with heterotrophic bacteria (either model bacteria or natural bacterial communities) are being conducted to assess the effects of climate change variables on metabolite transfer from phytoplankton to marine bacteria. In the first experiment type, bacteria are co-cultured with the phytoplankton at different temperatures and CO2 concentrations, and changes in bacterial gene expression and metabolite concentrations are used to assess shifts in the composition of metabolites transferred. In the second type, bacteria are grown on phytoplankton metabolite pools produced at different temperatures and CO2 concentrations in high-throughput bioassays, and changes in bacterial traits (growth rate, carrying capacity, growth efficiency) resulting from the different climate scenarios are used to indicate changes in metabolite quality. Knowledge of how the heterotrophic processing of phytoplankton metabolites might shift in response to climate change allows better prediction of Earth's future carbon cycle.

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.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1948104

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