High throughput growth screening of the marine bacterium Ruegeria pomeroyi DSS-3 knockout mutants on 70 environmentally relevant marine substrates done in 2021.

Website: https://www.bco-dmo.org/dataset/904246
Data Type: experimental
Version: 1
Version Date: 2023-07-17

Project
» Function and Importance of Marine Bacterial Transporters of Plankton Exometabolites (C-CoMP Marine Bacterial Transporters)

Program
» Center for Chemical Currencies of a Microbial Planet (C-CoMP)

Contributors	Affiliation	Role
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Abstract
An array of transporter knockout mutants of the marine bacterium Ruegeria pomeroyi DSS-3 mutants were screened for growth on 70 environmentally relevant marine substrates. The array contains 156 isolated knockout mutants of putative transporter genes as well as positive and negative growth controls and is distributed across two 96-well plates. Actively growing cultures were inoculated into freshly prepared 96 well plates containing minimal medium with a single substrates as sole carbon source. Plates were grown for 24 to 96 hours with growth monitored by optical density at 600 nm. Mutants that demonstrated growth defects on a given substrate, relative to typical growth on that substrate were selected for higher resolution follow up testing.

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Coverage

Temporal Extent: 2022-07-07 - 2022-09-29

Methods & Sampling

The data were generated from laboratory experiments performed at the University of Florida, Gainesville, Florida.

Mutant cultures were pre-grown overnight in ½ YTSS medium with 50 mg ml-1 kanamycin. Screens were performed in L1 minimal medium (Guillard and Hargraves 1993) modified to a salinity of 20 ppt and amended with ammonium (3 mM), kanamycin (50 mg ml-1), and phosphorus as PO43- at 36 mM. Overnight cultures of individual mutants (2 ml) were inoculated into 198 ml of modified L1 with a single substrate as the sole carbon source at 8 mM carbon. Plates were incubated at 25oC with shaking, and optical density (OD600) was read at intervals of 6-24 h until cultures entered stationary phase at ~24-72 h (SpectraMax M3, Molecular Devices, San Jose, CA). Mutants exhibiting phenotypes in the initial screen were moved to the targeted screen.

Data Processing Description

Minimal data processing was performed. Data were imported into Excel merged and reformatted in R version 4.2.2.

A supplemental file is provided with alternate formatting, see supplemental file description for more information.

BCO-DMO Processing Notes:
* Added year to date column
* Converted date column to ISO format
* Removed row number column

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Data Files

File
High Throughput Growth Screening filename: 904246_v1_hts.csv (Comma Separated Values (.csv), 8.45 MB) MD5:a9347feb56f410a34a8dba636a9d2889 Primary data file for dataset 904246, version 1.

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Supplemental Files

File
High Throughput Growth Screening Data, alternat format filename: HighThroughputScreenForBCODMO3_17.xlsx (Microsoft Excel, 1.32 MB) MD5:ceeae7fb88cc0aff9a73322dacaeada8 Data were imported into Excel and manually formatted such that: each sheet provides results of the screens begun on a single date. The plate reader OD600 data are presented in blocks of 8 rows by 12 columns, representing the layout of a 96 well plate. Each column of blocked data represents data from a time point, given in the first row of the sheet. Each row of blocked data represents one plate on one substrate, given in the first column of the sheet. Cells are conditionally colored based on value (blue low, red high), so that wells with growth defect can be readily observed.
Mutant plate-well key filename: Mutants_plate_well_key.xlsx (Microsoft Excel, 16.52 KB) MD5:5d396acf8e28183687f0718ad7c16898 Identifies the mutant strain in each well position of each plate. Columns indicate the plate number, well location in 96 well plate, locus tag of the disrupted gene, initial genome annotation of the gene, and the location within the gene of the transposon insertion. Column names and descriptions: Plate: ID of arrayed mutant plate. Well: the row and column location of the well in the 96 well plate. Gene: locus tag of disrupted gene Protein_previous_name: existing gene annotation at initiation of experiment, some annotations have changed. Tn_Insertion_as_fraction_of_gene_length: location of transposon insertion within gene

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Related Publications

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

Schroer, W. F., Kepner, H. E., Uchimiya, M., Mejia, C., Rodriguez, L. T., Reisch, C. R., & Moran, M. A. (2023). Function and Importance of Marine Bacterial Transporters of Plankton Exometabolites. https://doi.org/10.1101/2023.01.19.524783
Results

Schroer, W. F., Kepner, H. E., Uchimiya, M., Mejia, C., Rodriguez, L. T., Reisch, C. R., & Moran, M. A. (2023). Functional annotation and importance of marine bacterial transporters of plankton exometabolites. ISME Communications, 3(1). https://doi.org/10.1038/s43705-023-00244-6
Results

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Parameters

Parameter	Description	Units
date	date of experiment initiation in ISO Format	unitless
substrate	substrate screened as sole carbon source	unitless
plate	ID of the arrayed mutant plate used	unitless
time	time (hours) of sample	hours (hrs)
well_row	row ID of well in 96 well plate	unitless
well_col	column ID of well in 96 well plate	unitless
OD	optical density read at 600 nm	nanometer (nm)

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Instruments

Dataset-specific Instrument Name	SpectraMax M3 plate reader, Molecular Devices, San Jose, CA
Generic Instrument Name	plate reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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Project Information

Function and Importance of Marine Bacterial Transporters of Plankton Exometabolites (C-CoMP Marine Bacterial Transporters)

Project:

Observing expression of marine bacterial transporter systems with transcriptomic or proteomic tools can provide valuable information about the metabolomic environment. However, these ‘omics approaches are limited by the low rate of transporter gene annotation. Here, a barcoded, arrayed, mutant library of the marine bacterium *Ruegeria pomeroyi* DSS-3 is employed in high throughput screens to identify the target substrates of 13 transporter systems. A set of 156 isolated putative transporter mutants were screened for growth on minimal medium with 63 substrates, each as a sole carbon source. Mutants that demonstrated a growth defect on a specific substrate were selected for secondary, higher resolution, growth screening. Mutants that continued to demonstrate growth defect relative to the pooled-mutant library (pooled-BarSeq, used as an analog for wildtype) were screened for their ability to drawdown the target substate. Gene annotations were made when mutants of the given transporter demonstrated both growth and drawdown defects on the target substrate.

In addition to the isolated mutant screens, the pooled barcoded transposon mutant library (pooled-BarSeq) was grown on minimal medium with selected substrates, each as sole carbon source, such that the relative enrichment or depletion of each mutant could demonstrate its fitness cost associated with the loss of each disrupted gene when grown on each substrate. The results of pooled-BarSeq screens had mixed consistency with the isolated mutant screens, demonstrating the value of isolated mutants for transporter annotation.

Program:

The Center for Chemical Currencies of a Microbial Planet (C-CoMP) integrates research, education and knowledge transfer activities to develop a mechanistic understanding of surface ocean carbon flux within the context of a changing ocean and through increased participation in ocean sciences. C-CoMP supports science teams that merge biology, chemistry, modeling, and informatics to close long-standing knowledge gaps in the identities and dynamics of organic molecules that serve as the currencies of elemental transfer between the ocean and atmosphere.

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Program Information

Center for Chemical Currencies of a Microbial Planet (C-CoMP)

Website: https://ccomp-stc.org/

Coverage: North Atlantic, BATS, global/other

Functions carried out by microscopic inhabitants of the surface ocean affect every aspect of life on our planet, regardless of distance from the coast. Ocean phytoplankton are responsible for half of the photosynthesis on Earth, the first step in a complex system that annually withdraws 50 billion metric tons of carbon from the atmosphere to sustain their growth. Of this, 25 billion metric tons participate in a rapid cycle in which biologically reactive material is released into seawater and converted back into carbon dioxide by marine bacteria within hours to days. The chemical-microbe network at the heart of this fast cycle remains poorly constrained; consequently, its primary currencies and controls remain elusive; its sensitivities to changing ocean conditions are unknown; and its responses to future climate scenarios are not predictable. The Center for Chemical Currencies of a Microbial Planet (C-CoMP) integrates research, education and knowledge transfer activities to develop a mechanistic understanding of surface ocean carbon flux within the context of a changing ocean and through increased participation in ocean sciences. C-CoMP supports science teams that merge biology, chemistry, modeling, and informatics to close long-standing knowledge gaps in the identities and dynamics of organic molecules that serve as the currencies of elemental transfer between the ocean and atmosphere. C-CoMP fosters education, outreach, and knowledge transfer activities that engage students of all ages, broaden participation in the next generation of ocean scientists, and extend novel open-science approaches into complementary academic and industrial communities. The Center framework is critical to this mission, uniquely facilitating an open exchange of experimental and computational science, methodological and conceptual challenges, and collaborations that establish integrated science and education partnerships. With expanded participation in ocean science research and ocean literacy across the US society, the next generation of ocean scientists will better reflect the diverse US population.

Climate-carbon feedbacks on the marine carbon reservoir are major uncertainties for future climate projections, and the trajectory and rate of ocean changes depend directly on microbial responses to temperature increases, ocean acidification, and other perturbations driven by climate change. C-CoMP research closes an urgent knowledge gap in the mechanisms driving carbon flow between ocean and atmosphere, with global implications for predictive climate models. The Center supports interdisciplinary science teams following open and reproducible science practices to address: (1) the chemical currencies of surface ocean carbon flux; (2) the structure and regulation of the chemical-microbe network that mediates this flux; and (3) sensitivity of the network and its feedbacks on climate. C-CoMP leverages emerging tools and technologies to tackle critical challenges in these themes, in synergy with existing ocean programs and consistent with NSF's Big Ideas. C-CoMP education and outreach activities seek to overcome barriers to ocean literacy and diversify participation in ocean research. The Center is developing (1) initiatives to expand ocean literacy in K-12 and the broader public, (2) ocean sciences undergraduate curricula and research opportunities that provide multiple entry points into research experiences, (3) post-baccalaureate programs to transition undergraduates into graduate education and careers in ocean science, and (4) interdisciplinary graduate student and postdoctoral programs that prepare the next generation of ocean scientists. The C-CoMP team includes education faculty who evaluate the impacts of education and outreach activities and export successful STEM initiatives to the education community. C-CoMP is revolutionizing the technologies for studying chemical transformations in microbial systems to build understanding of the outsized impact of microbes on elemental cycles. Open science, cross-disciplinary collaborations, community engagement, and inclusive practices foster strategic advances in critical science problems and STEM initiatives. C-CoMP science, education, and knowledge-transfer themes are efficiently addressed through a sustained network of scientists addressing critical research challenges while broadening the workforce that will tackle multi-disciplinary problems with academic, industrial and policy partners.

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.

The Program's Data Management Plan (DMP) is available as a PDF document.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2019589
Simons Foundation (Simons)	542391

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