Incubation experiments were conducted in St. John, US Virgin Islands to investigate the response of reef seawater microbial communities to the specific metabolites riboflavin, pantothenic acid, and caffeine.

Website: https://www.bco-dmo.org/dataset/865159
Data Type: experimental
Version: 1
Version Date: 2022-11-10

Project
» Signature exometabolomes of Caribbean corals and influences on reef picoplankton (Coral Exometabolomes)

Contributors	Affiliation	Role
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Abstract
Pre-filtered reef seawater microbial communities collected from Lameshur Bay, U.S. Virgin Islands were incubated separately in the presence of the individual metabolites riboflavin, pantothenic acid, and caffeine for 24 hours and samples were collected to monitor changes in microbial community composition using 16S rRNA gene sequencing and microbial abundances using flow cytometry. Targeted metabolomic data from these incubations is available on the MetaboLights database under accession number MTBLS3286.

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Coverage

Spatial Extent: **Lat**:18.315773476841 **Lon**:-64.727413483487

Dataset Description

Note: This flow cytometry data is the raw data

Other relevant files and publications:

The targeted and untargeted metabolomics data and metadata associated with this study are located on the MetaboLights database under accession numbers MTBLS2855 and MTBLS3286.

The 16S rRNA gene sequencing data and metadata associated with this study are located on the NCBI Sequence Read Archive (SRA) under BioProject PRJNA739882. BioSample accession numbers are not linked with the data submitted to BCO-DMO because samples for flow cytometry and macronutrients were not always collected at the same time as samples collected for microbial community analyses, meaning that only

some of the samples collected for microbial community analyses have affiliated microbial abundances and nutrient concentrations.

Methods & Sampling

Three metabolite uptake incubation experiments were conducted in January 2021 based on the results from previous incubations. These experiments were designed to assess if reef seawater microbial communities assimilate specific exometabolites released by corals and if the microbial communities respond distinctly to different metabolites. Experiments were conducted with three metabolites in separate incubations occurring on different days: riboflavin, pantothenic acid, and caffeine. Riboflavin and pantothenic acid were chosen because both metabolites were released by at least 4/6 species within the organism incubation experiments and because both are B vitamins. Caffeine was selected because it was released in high quantities by *R. textilis*. Samples were collected at three time points over the course of the incubation: 0, 6, and 24 hrs. Additionally, incubations were conducted in the dark and processed in low-light conditions to minimize photodegradation of the metabolites, especially riboflavin.

Before the incubations were conducted, purified metabolite standards were diluted with MQ water to 5 nM (riboflavin, D-pantothenic acid) and 10 nM (caffeine) one month prior to the incubations, frozen, and shipped to the USVI. Metabolite dilutions were prepared in combusted amber vials and kept in the dark to minimize photochemical degradation. The incubations were conducted in St. John, U. S. Virgin Islands from January 16th-January 21st, 2021.

To set up the incubations, 5.5 l of seawater was collected approximately 1 m above the reef at Tektite reef (9 m depth) using Niskin bottles deployed by divers. Half of the seawater was filtered using a 0.1 µm, 47 mm Omnipore filter to create filtered seawater (FSW), while the rest of the seawater was filtered using a 1 µm, 47 mm Omnipore filter in order to remove larger phytoplankton and protistan grazers, but retain the picoplanktonic microbial community. The filtrate treatments were poured into 36 acid-washed and autoclaved 125 ml polycarbonate bottles. Due to the small volumes and to minimize the chance of sample contamination, the incubations were designed so that bottles could be sampled by sacrifice at each of the three timepoints. Each incubation had four different experimental conditions: 3 different controls and one experimental treatment. The three controls included only FSW (F), FSW with the addition of a metabolite spike (F + Mtb), and 1 µm filtered seawater containing microbes (M). The experimental condition was 1 µm filtered seawater containing microbes with the addition of the metabolite (M + Mtb). Metabolite spikes (20 pM for riboflavin and pantothenic acid and 70 pM for caffeine) were added to the FSW + Mtb and M + Mtb treatments by removing 500 µl (riboflavin and pantothenic acid incubations) or 875 µl (caffeine incubation) of seawater from each incubation bottle, replacing the lost volume with the appropriate metabolite spike, and inverting the bottles to mix. After the metabolite spikes were added, 24 of the 36 incubation bottles were placed in a flow-through seawater table covered with a tarp and equipped with a HOBO data logger to monitor relative light levels and water temperature, while the remaining 12 bottles, accounting for triplicate bottles of each of the four experimental conditions, were immediately processed for the initial timepoint.

After each time point, samples from the incubation bottles were directly collected for assessment of microbial biomass via flow cytometry or re-filtered using 0.2 µm, 47 mm Omnipore filters. The filters were placed into cryovials, frozen in a dry shipper, and the filtrate was collected.

Data Processing Description

Microbial abundances of *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and unpigmented cells were enumerated via flow cytometry. Briefly, samples were thawed and stained with Hoechst 34442 (1 µg/mL, final concentration) [1–3] and analyzed using a Beckman-Coulter Altra Flow Cytometer endowed with two argon ion lasers, tuned to UV (200 mW) and 488 nm (1 W) excitation wavelengths. Side and forward scatter as well as fluorescence signals were collected using the appropriate filters designated for Hoechst-bound DNA, phycoerythrin, and chlorophyll. FlowJo software (Tree Star, Inc.) was used to bin populations and estimate the abundances of *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and unpigmented cells (primarily heterotrophic bacteria and archaea). Trends in microbial abundances of *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and unpigmented cells (~ heterotrophic bacteria and archaea) yielded via flow cytometry were inspected using line graphs and standard error was calculated across replicate samples for each time point.

To generate the sequencing library for microbial community analysis, DNA was extracted from the 47 mm 0.2

µm filters using the PowerBiofilm DNA extraction kit following the standard protocol. Seven DNA extraction controls were created to control for contamination. Amplification was conducted with the 515F-Y [7] and 806R-B [8] primers and sequencing occurred using Illumina MiSeq 2x250 bp. Microbial community analysis was completed using the methods outlined above for the exudate uptake experiments. The data is available on NCBI Sequence Read Archive (SRA) under BioProject PRJNA739882.

The methods and data files generated from the metabolite uptake incubations collections will be available on the MetaboLights database under accession number MTBLS3286. This submission is currently under curation at MetaboLights.

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Data Files

File
metabo_uptake.csv (Comma Separated Values (.csv), 4.29 KB) MD5:86fc31755d9ef2df99a3cf1b0b99d174 Primary data file for dataset ID 865159

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Related Publications

Apprill, A., & Rappé, M. (2011). Response of the microbial community to coral spawning in lagoon and reef flat environments of Hawaii, USA. Aquatic Microbial Ecology, 62(3), 251–266. doi:10.3354/ame01471
Methods

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137. doi:10.3354/ame01753
Methods

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
Methods

Campbell, L., & Vaulot, D. (1993). Photosynthetic picoplankton community structure in the subtropical North Pacific Ocean near Hawaii (station ALOHA). Deep Sea Research Part I: Oceanographic Research Papers, 40(10), 2043–2060. doi:10.1016/0967-0637(93)90044-4
Methods

Campbell, L., Nolla, H. A., & Vaulot, D. (1994). The importance of Prochlorococcus to community structure in the central North Pacific Ocean. Limnology and Oceanography, 39(4), 954–961. doi:10.4319/lo.1994.39.4.0954
Methods

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. Microbiome, 6(1). doi:10.1186/s40168-018-0605-2
Methods

Hansell, D. A., & Carlson, C. A. (2001). Biogeochemistry of total organic carbon and nitrogen in the Sargasso Sea: control by convective overturn. Deep Sea Research Part II: Topical Studies in Oceanography, 48(8-9), 1649–1667. doi:10.1016/s0967-0645(00)00153-3 https://doi.org/10.1016/S0967-0645(00)00153-3
Methods

Martin BD, Witten D, Willis AD (2019) Modeling microbial abundances and dysbiosis with beta-binomial regression. arXiv:1902.02776 [stat].
Methods

Monger, B. C., & Landry, M. R. (1993). Flow Cytometric Analysis of Marine Bacteria with Hoechst 33342 †. Applied and Environmental Microbiology, 59(3), 905–911. doi:10.1128/aem.59.3.905-911.1993
Methods

Oksanen J. Vegan: ecological diversity. R Packag Version 24-4 . 2017. https://cran.r-project.org/src/contrib/Archive/vegan/vegan_2.4-4.tar.gz
Software

Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2015). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology, 18(5), 1403–1414. doi:10.1111/1462-2920.13023 https://doi.org/http://doi.org/10.1111/1462-2920.13023
Methods

Weber, L., Gonzalez-Díaz, P., Armenteros, M., & Apprill, A. (2019). The coral ecosphere: A unique coral reef habitat that fosters coral–microbial interactions. Limnology and Oceanography, 64(6), 2373–2388. doi:10.1002/lno.11190
Methods

Weber, L., Soule, M. K., Longnecker, K., Becker, C. C., Huntley, N., Kujawinski, E. B., & Apprill, A. (2022). Benthic exometabolites and their ecological significance on threatened Caribbean coral reefs. ISME Communications, 2(1). https://doi.org/10.1038/s43705-022-00184-7
Results

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Parameters

Parameter	Description	Units
Sample_ID	Sample ID	unitless
Filtration_type	Filtration type: 1um_seawater:reef seawater filtered through 1 µm filter, FSW: reef seawater filtered through - 0.1 µm filter	unitless
Time	Time point: T0, T6 or T24	unitless
Treatment	Treatment: M+Mtb: Microbes+metabolite, F+Mtb: Filtered Seawater + metabolite, F: Filtered Seawater only, M: Microbes only	unitless
Incubation	Individual metabolite: riboflavin, pantothenic acid, and caffeine	unitless
Prochlorococcus	Prochlorococcus abundance	cells per milliliter (cells/ml)
Synechococcus	Synechococcus abundance	cells per milliliter (cells/ml)
Picoeukaryotes	Picoeukaryote abundance	cells per milliliter (cells/ml)
Unpigmented_cells	Unpigmented cells abundance	cells per milliliter (cells/ml)

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Instruments

Dataset-specific Instrument Name	Illumina sequencers
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina iSeq 100 sequencer Illumina MiSeq sequencer (2x250 bp)
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	Shimadzu TOC-VCSH total organic carbon analyzer with a TNM-1 module
Generic Instrument Name	Shimadzu TOC-V Analyzer
Dataset-specific Description	Shimadzu TOC-VCSH total organic carbon analyzer with a TNM-1 module
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

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Project Information

Signature exometabolomes of Caribbean corals and influences on reef picoplankton (Coral Exometabolomes)

Coverage: U.S. Virgin Islands

NSF Award Abstract:
Coral reefs are some of the most diverse and productive ecosystems in the ocean. Globally, reefs have declined in stony (reef-building) coral abundance due to environmental variations, and in the Caribbean this decline has coincided with an increase in octocoral (soft coral) abundance. This phase shift occurring on Caribbean reefs may be impacting the interactions between the sea floor and water column and particularly between corals and picoplankton. Picoplankton are the microorganisms in the water column that utilize organic matter released from corals to support their growth. These coral-picoplankton interactions are relatively unstudied, but could have major implications for reef ecology and coral health. This project will take place in the U.S. territory of the Virgin Islands (USVI) and will produce the first detailed knowledge about the chemical diversity and composition of organic matter released from diverse stony coral and octocoral species. This project will advance our understanding of coral reef microbial ecology by allowing us to understand how different coral metabolites impact picoplankton growth and dynamics over time. The results from this project will be made publically accessible in a freely available online magazine, and USVI minority middle and high school students will be exposed to a lesson about chemical-biological interactions on coral reefs through established summer camps. This project will also contribute to the training of USVI minority undergraduates as well as a graduate student.

Coral exometabolomes, which are the sum of metabolic products of the coral together with its microbiome, are thought to structure picoplankton communities in a species-specific manner. However, a detailed understanding of coral exometabolomes, and their influences on reef picoplankton, has not yet been obtained. This project will utilize controlled aquaria-based experiments with stony corals and octocorals, foundational species of Caribbean reef ecosystems, to examine how the exometabolomes of diverse coral species

differentially influence the reef picoplankton community. Specifically, this project will capitalize on recent developments in mass spectrometry-based metabolomics to define the signature exometabolomes of ecologically important and diverse stony corals and octocorals. Secondly, this project will determine how the exometabolomes of these corals vary with factors linked to coral taxonomy as well as the coral-associated microbiome (Symbiodinium algae, bacteria and archaea). With this new understanding of coral exometabolomes, the project will then apply a stable isotope probe labeling approach to the coral exometabolome and will examine if and how (through changes in growth and activity) the seawater picoplankton community incorporates coral exometabolomes from different coral species over time. This project will advance our ability to evaluate the role that coral exometabolomes play in contributing to benthic-picoplankton interactions on changing Caribbean reefs.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1736288

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