16S V4 rRNA gene tag sequences from reef seawater samples collected in the Florida Keys and the U.S. Virgin Islands in 2019-2020

Website: https://www.bco-dmo.org/dataset/858459
Data Type: Other Field Results
Version: 1
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Project
» EAGER: Collaborative Research: Quantifying coral microbiome dynamics under change (Quantitative coral microbiomes)

Contributors	Affiliation	Role
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Abstract
This dataset describes 16S V4 rRNA gene tag sequences from reef seawater. Samples were collected from three reefs in U.S. Virgin Islands and three reefs in Florida Reef tract. Sequence data can be found in the NCBI SRA database under the bioproject PRJNA733652.

Table of Contents

Coverage

Spatial Extent: **N**:25.38634 **E**:-64.980451 **S**:18.316767 **W**:-82.828323
Temporal Extent: 2019-06-07 - 2020-02-12

Acquisition Description

Samples were collected in two reef systems, the Florida Reef Tract in June of 2019 and off the southern coast of St. Thomas in the U.S. Virgin Islands in Februrary of 2020. See Supplemental File, "sampling_locations.csv" for latitude, longitude, depth, and dates of each sampling location. A total of 3 reef sites at each reef system were sampled. At each reef site, three 10 meter transects were taken by laying down a 10 meter weighted line that was marked every meter. Water samples were taken by a diver using a 60 or 100 mL syringe positioned approximately 5 mm above the benthos at each meter line. The transects were laid haphazardly, but did not intersect with each other. Because of inclement conditions, only 1 transect was collected at Site 73, rather than the intended 3. To capture the seawater microbial community, 60 mL of the seawater was filtered through a 0.22 µm Supor filter (25 mm; Pall Corporation).

Filters were placed in 2 mL cryovials, flash frozen in a liquid nitrogen dry shipper, and processed upon return to Woods Hole, MA.

DNA was extracted from the filters using the DNEasy PowerBiofilm Kit (Qiagen) according to manufacturer protocols. Seven DNA extraction controls, consisting of unused 0.22 µm filter, were processed alongside samples. Primers 515F (Parada et al. 2016) and 806R (Apprill et al. 2015) containing Nextera adapter sequences were used to amplify the V4 region of the small subunit rRNA gene in bacteria and archaea.

PCR reactions contained 14.75 µL molecular grade water, 5 µL GoTaq Flexi 5X buffer (Promega Corporation), 2.5 µL of 25 mM MgCl2, 1 µL of 10 mM dNTPs, 1 µL of 10 mM forward and reverse primers, 0.5 µL GoTaq DNA polymerase (Promega). Three PCR controls consisting of 1 µL of PCR-grade water was also included, as well as microbial genomic DNA from a Human Microbiome Project mock community (BEI Resources, NIAID, NIH as part of the Human Microbiome Project: Genomic DNA from Microbial Mock Community B (Even, Low Concentration), v5.1L, for 16S RNA Gene Sequencing, HM-782D). The first stage PCR conditions were: 28 cycles (95°C 20s, 55°C 20s, 72°C 5 min) with a 2 minute 95°C hot start and 10 min 72°C final elongation. PCR products were screened for quality using gel electrophoresis and purified using the MinElute PCR purification kit (Qiagen). PCR products were then barcoded using the Nextera XT Index Kit v2 primers (Illumina) using the following conditions: 8 cycles (95°C 30s, 55°C 30s, 72°C 30s) with 3 minute 95° hot start and 5 minute 72°C final elongation. Barcoded products were purified as above and concentrations of the purified products were assessed using the HS dsDNA assay on the Qubit 2.0 fluorometer (ThermoFisher Scientific). Products were diluted with Tris HCl to 5 nM before being pooled randomly into two libraries. The libraries were diluted to a final loading concentration of 50 pM with a 5% spike-in of 50 pM PhiX. The libraries were then sequenced on the iSeq 100 System (Illumina).

Processing Description

Sequences deposited on the NCBI database are raw sequence reads before primer trimming or any processing. Sequences can be found in NCBI under the bioproject PRJNA733652.

BCO-DMO Processing:
- replaced 'NA' with 'nd' (no data);
- changed date format to YYYY-MM-DD;
- renamed 'SRA_runk_link' to 'SRA_run_link';
- removed commas from geo_loc_name field.

[table of contents | back to top]

Related Datasets

IsRelatedTo

Woods Hole Oceanographic Institution. Biogeography of reef water microbes at transect, reef and global scales. 2021/05. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA733652. NCBI:BioProject: PRJNA733652.

[table of contents | back to top]

Parameters

Parameter	Description	Units
BioProject	NCBI BioProject number	unitless
Sample_Name	Sample identifier	unitless
SRA_Run_ID	SRA run ID	unitless
SRA_run_link	SRA run link	unitless
SRA_Study	SRA study ID	unitless
SRA_Sample	SRA sample	unitless
geo_loc_name	Geolocation name	unitless
isolation_source	Description of source of sample	unitless
lat	Latitude of sample collection	unitless
lon	Longitude of sample collection	unitless
Assay_Type	Assay type	unitless
Platform	Instrument platform	unitless
Instrument	Instrument	unitless
LibraryLayout	Description of library layout	unitless
LibrarySelection	Library selection	unitless
LibrarySource	Library source	unitless
Organism	Organism descriptoin	unitless
samp_collect_device	Sample collection device	unitless
samp_mat_process	Sample extraction process	unitless
reef_system	Reef system: FLA = Florida Keys; STT = St. Thomas	unitless
site	Site identifier	unitless
substrate_class	Description of substrate	unitless
transect	Transect number	unitless
distance	Distance along transect	meters
Sample_or_Control	Indicates if the sample is a control	unitless
collection_date	Date of sample collection; format: YYYY-MM-DD	unitless
unique_ID	Unique ID for each row	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Qubit 2.0 fluorometer (ThermoFisher Scientific)
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	diver and syringe
Generic Instrument Name	Manual Biota Sampler
Dataset-specific Description	Water samples were taken by a diver using a 60 or 100 mL syringe
Generic Instrument Description	Manual Biota Sampler indicates that a sample was collected in situ by a person, possibly using a hand-held collection device such as a jar, a net or their hands.

Dataset-specific Instrument Name	iSeq 100 System (Illumina)
Generic Instrument Name	Automated DNA Sequencer
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	PCR
Generic Instrument Name	PCR Thermal Cycler
Generic Instrument Description	General term for a laboratory apparatus commonly used for performing polymerase chain reaction (PCR). The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

[table of contents | back to top]

Project Information

EAGER: Collaborative Research: Quantifying coral microbiome dynamics under change (Quantitative coral microbiomes)

Coverage: Little Cayman

NSF Award Abstract:
Corals are the architectural base of reefs, one of the most biodiverse and ecologically complex ocean ecosystems that also provide substantial economic resources to coastal communities. Unfortunately, recent episodes of widespread bleaching and disease have reduced coral populations and contributed to declines in reef ecosystems. There is speculation that the coral microbiome, including a community of bacteria and archaea, may provide added resistance and resilience to corals facing pathogens and warmer ocean conditions. However, there are no quantitatively methods available to track specific microbial lineages within coral microbiomes, thus limiting the ability to examine these concepts. This research will improve capabilities to quantitatively measure and track specific microbial lineages within the coral microbiome. Coral reefs are one of the most globally threatened ecosystems in the ocean. The project will provide methodological advancements that will enhance understanding about the response of coral microbiomes to warming, disease and environmental-related disturbance. These efforts will help provide a necessary knowledge framework for scientists, reef managers and decision makers who are urgently exploring solutions to prevent the further decline of coral reef ecosystems. The team will communicate project findings broadly through publicly accessible narratives shared through press releases and an online magazine. This project will promote data sharing and collaboration amongst coral microbiome scientists, through a data sharing and visualization portal. The project will train undergraduate and graduate students and will be led by two female PIs (one Hispanic).

This research has the potential to transform our current ability to quantify ecological changes within coral microbiomes and to understand and predict how coral-associated microbes may be able to contribute to the resilience and resistance of corals to warming, disease and environmental change related stressors on reefs. This project will contribute methodological and resource contributions that will advance knowledge and studies of coral microbiomes as well as other host-microbiome systems. Specifically, the team plans to develop and test two types of controls, 1) spike-in microbial cells and 2) a coral microbiome specific mock community. These methods will be used to address the following hypothesis: improved quantitative descriptions of coral microbiome dynamics in relation to relevant scenarios of change are obtained using spike-in controls and coral-specific mock communities. To address this, the investigators will develop endogenous spike-in controls of bacteria and archaea to track microbial load or abundance within samples and construct a coral microbiome mock community to optimize PCR, sequencing and data analysis. These methods will first be optimized in a replicated experimental manner across healthy, nonstressed and ecologically and phylogenetically diverse coral species. Next, optimized methods will be applied to coral samples from experiments, which are expected to show changes in microbiome dynamics over time. These experiments include scenarios of thermal stress, reef environmental change, and disease development. These experiments will take place in Little Cayman, a location with reefs with contrasting temperature and other environmental properties, as well as the U.S. Virgin Islands, a site of a recent coral disease outbreak.

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.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1938147

[table of contents | back to top]