Illumina sequenced 16S rRNA genes amplified from the different life stages of 2 corals in Kaneohe Bay, Oahu, Hawaii during 2010 (MiCoDe project)

Website: https://www.bco-dmo.org/dataset/553168
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Project
» The Development of Microbial Associations in Major Reef Building Corals of the Pacific Ocean (MiCoDe)

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Dataset Description

Illumina sequenced 16S rRNA genes amplified from different life stages of the corals *Fungia scutaria* and *Montipora capitata* from central Kaneohe Bay off of the island of Oahu, Hawaii.

Methods & Sampling

Fungia scutaria sample collection

Adult specimens of the solitary coral *F. scutaria* were collected from Kaneohe Bay on the northeastern shore of Oahu, Hawaii in the Pacific Ocean in June 2010 and kept in open-system tanks (sea tables) at the Hawaii Institute of Marine Biology in anticipation of spawning events. Following spawning, eggs and sperm were collected for fertilization in Petri dishes, and replicate batches of larvae were raised in 0.1 µm-filtered (sterile), 1.6 µm-filtered (excludes *Symbiodinium* and zooplankton), 20 µm-filtered (excludes zooplankton) or raw seawater. Samples from each life history stage (eggs, larvae, juveniles, and adults) were frozen for DNA analysis in DNA lysis buffer. Eggs and embryos were rinsed in sterilized seawater upon sampling. Each adult sample consists of three small pieces of an individual coral combined in one tube. Ca. 1 L of seawater was sampled from the sea tables one hour prior to the final spawning event, filtered, and frozen for DNA analysis in DNA lysis buffer. 50 mL of the culture water was sampled from the Petri dish treatments (originally 0.1 µm-filtered, 1.6 µm-filtered, or 20 µm-filtered seawater) after approximately 72 hours of exposure to the developing coral larvae during the final experiment.

Montipora capitata sample collection

Fragments of adult specimens of the colonial coral *M. capitata* were collected from Kaneohe Bay in June 2010 and July 2011 and kept in sea tables at HIMB in anticipation of spawning events. Following spawning, egg-sperm bundles were collected from the sea tables for fertilization in Petri dishes, and replicate batches of larvae were raised in 0.1 µm-filtered, 1.6 µm-filtered, or 20 µm-filtered seawater. Samples from each life history stage

(egg-sperm bundles, larvae, juveniles, and adults) were frozen for DNA analysis in DNA lysis buffer. Egg-sperm bundles were rinsed in TFF sterilized seawater upon sampling. Each adult sample consists of three small pieces of an individual coral combined in one tube. Ca. 1 L of seawater was sampled from the sea tables approximately one hour prior to the final spawning event, filtered, and frozen for DNA analysis in DNA lysis buffer. 35 mL of the culture water was sampled from the Petri dish treatments (originally 0.1 µm-filtered, 1.6 µm-filtered, or 20 µm-filtered seawater) after approximately 72 hours of exposure to the developing coral larvae during the final experiment in 2010, filtered, and frozen for DNA analysis in DNA lysis buffer. Additionally, throughout summer 2010, field-spawned egg-sperm bundles and field-fertilized larvae were collected from Kaneohe Bay and raised under the same treatments (0.1 µm-filtered, 1.6 µm-filtered, 20 µm-filtered, or raw seawater), and sampled in the same manner and at the same life-history stages (egg-sperm bundles, larvae, juveniles, and adults) as the corals spawned in sea tables. Ca. 1 L of seawater was sampled from the Kaneohe Bay lagoon 1 hour prior to the August 2010 field-spawning event and 1 hour after the June and July 2010 field-spawning events, filtered, and filters were frozen for DNA analysis in DNA lysis buffer.

DNA extraction and Illumina amplicon sequencing of bacterial SSU rRNA genes

Nucleic acids were extracted from *F. scutaria* corals and filters using the NucleoSpin Soil Kit (Macherey-Nagel, Duren, Germany) following the manufacturer's protocol, modified as follows: lysis buffer SL1 and Enhancer SX were used, lysis time was increased to 20 min and two subsequent elution steps were performed with 50 µl fresh elution buffer each. DNA quality and yield were checked by agarose gel electrophoresis.

Nucleic acids were extracted from *M. capitata* samples and filters using the PowerSoil DNA Isolation Kit (MO BIO, Carlsbad, CA) following the manufacturer's protocol, modified as follows: initial physical disruption time (via vortex) was increased to 30 min, and two subsequent elution steps were performed with 100 µl fresh DNA-Free PCR Grade water each. DNA quality and yield were checked by agarose gel electrophoresis.

The V4 region of the 16S rRNA gene was amplified with the primer pair F515/R806, which include Illumina flowcell adapter sequences and a sample-specific barcode on the reverse primer. The MasterTaq Kit (5 PRIME, Hamburg, Germany) was used for all PCR reactions. Amplification was performed using a touchdown protocol as in as follows: initial denaturation at 95°C for 3 min, and 30 cycles at 95°C for 30 sec, 65°C for 1 min (decreasing by 0.5°C per cycle), and 72°C for 2 min. After these 30 cycles, a further 5 – 7 cycles were performed using an annealing temperature of 50°C, and concluding with a final extension at 72°C for 20 min. Resulting PCR amplicons were quantified using the PicoGreen dsDNA assay (Invitrogen, Carlsbad, CA), pooled at equimolar concentrations and subsequently cleaned using the UltraClean PCR Clean-Up Kit (MO BIO Laboratories, Carlsbad, CA). Sequencing of the *F. scutaria* PCR products was performed on an Illumina MiSeq at the University of Colorado BioFrontiers Institute (Boulder, CO), while sequencing of the *M. capitata* PCR products was performed on an Illumina MiSeq at the University of Hawaii HIMB Genetics Core Facility (Kaneohe, HI).

Data Processing Description

BCO-DMO Processing:

original file: Shulse_Illumina_2015_metadata_V2.xls
- added conventional header with dataset name, PI name
- renamed parameters to BCO-DMO standard
- reformatted date from d-Mon-yy to yyyy-mm-dd
- replaced NA with nd
- replaced spaces with underscores
- replaced special characters: = lteq
- reordered columns
- sorted date by species, sample_type, sample_id

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Data Files

File
illumina_2015_sort.csv (Comma Separated Values (.csv), 86.63 KB) MD5:2a0ba10a8e81bfe3b9b9037e39753b08 Primary data file for dataset ID 553168

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Parameters

Parameter	Description	Units
species	species of coral	unitless
sample_type	developmental stage	unitless
treatment	conditions including filtration size of seawater	unitless
sample_id	sample identification	unitless
date_coll	date collected	yyyy-mm-dd
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
BioSample	GenBank BioSample id	unitless
SRA_accession	GenBank SRA_accession id	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina MiSeq desktop 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	Thermal Cycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA)
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. 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. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

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Deployments

Rappe_2010

Website	https://www.bco-dmo.org/deployment/553234
Platform	Hawaii_reef
Start Date	2010-06-01
End Date	2010-06-30
Description	coral sampling for lab experiments

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Project Information

The Development of Microbial Associations in Major Reef Building Corals of the Pacific Ocean (MiCoDe)

Website: http://www.soest.hawaii.edu/oceanography/faculty/rappe/RappeLab/CAM.html

Coverage: Kaneohe Bay, HI, USA; and Cook’s Bay, Moorea, French Polynesia

Description from NSF award abstract:
Fundamental to the study of coral-microbial associations is an understanding of when and how the relationships are established, and their specificity. Recently, the investigators provided the first evidence of a specific association between an early life history stage of a coral (*Pocillopora meandrina*) and a particular group of bacteria (Jannaschia lineage of the Roseobacter clade of Alphaproteobacteria). They will be expanding this work by examining the onset of microbial associations in key reef building corals from Hawaii in the North Pacific Ocean and Moorea, French Polynesia, in the South Pacific Ocean. Understanding the onset, specificity and function of the microbial community associated with these coral species is necessary to understand and predict the coral holobiont response to a changing environment.

The main objectives of this proposal are to:

1. Use cultivation independent techniques to identify and quantify microorganisms associated with several major reef building corals of Hawaii in the North Pacific Ocean that represent a variety of reproductive strategies (brooding and broadcast spawning) and differing modes of zooxanthellae symbiont transmission (vertical vs. horizontal), throughout the reproductive cycle, early developmental stages, and post-settlement stages of each.
2. Collect and analyze similar samples from the same (or similar) species of coral found in the South Pacific Ocean in Moorea, French Polynesia, in order to assess whether the associations documented in objective 1 are localized to Hawaii, or broadly distributed across the Pacific and likely to represent common features of coral development.
3. Use fluorescence in situ hybridization to enumerate cells of the Jannaschia lineage of the Roseobacter clade throughout the development cycle of P. meandrina collected in Hawaii. The PIs will expand this objective to include other coral species, target bacteria, and/or geographic location as they identify additional associations.
4. Isolate microorganisms prevalent in cultivation-independent surveys of P. meandrina-associated microbial communities (e.g. Jannaschia sp.) by the application of novel culturing techniques, in order to develop model systems for the investigation of coral-microbe interactions.

Coral reefs are in decline as a result of increasing environmental stress due to anthropogenic activity, and there is now considerable evidence indicating that they are under threat from the effects of rising sea surface temperature and ocean acidification. Microorganisms associated with corals are thought to play a variety of potentially important roles in maintaining the health and resiliency of the coral host, and advances in methodology primarily driven by developments in the field of molecular biology are facilitating growing insight into this association. Much of coral-microbial research is focused on the contribution of microorganisms to disease and bleaching, and is focused almost exclusively on adult coral colonies. This study will provide unique information on the manner in which microorganisms interact with healthy corals throughout their developmental cycle, the specificity of these relationships, how they are initiated, and their distribution and frequency in nature.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0928806

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