Hawaiian coral terminal-restriction fragment length polymorphism (T-RFLP) analysis collected from the R/V Hi'ialakai in Hawaiian Archipelago, Johnston Atoll, and American Samoa during 2005 (MiCoDe project)

Website: https://www.bco-dmo.org/dataset/553101
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Project
» The Development of Microbial Associations in Major Reef Building Corals of the Pacific Ocean (MiCoDe)

Contributors	Affiliation	Role
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Dataset Description

Molecular biogeography of seawater and coral-associated microorganisms across the Hawaiian Archipelago.

Methods & Sampling

During the September/October 2005 cruise, samples were collected from multiple reef sites around Necker Island, French Frigate Shoals, Maro Reef, Pearl and Hermes Atoll, Midway Atoll, and Kure Atoll. During the May/June 2006 cruise, samples were collected from Kaneohe Bay (Oahu), Nihoa, French Frigate Shoals, Gardner Pinnacles, Johnston Atoll, and American Samoa.

Coral colonies judged as non-diseased by visual inspection were sampled. A stainless steel chisel was used to remove three dime-sized pieces of coral from each colony that consisted of coral tissue, overlying mucous layer, and underlying skeleton. The three sub-samples from each colony were put into sterile bags (Whirl-pak; Nasco, Fort Atkinson, WI), placed on ice, and transported back to the ship where they were frozen. Temperature and depth below sea surface were recorded at each site with a dive computer.

Seawater samples were also collected to characterize planktonic bacterial communities in the surrounding environment. One liter of seawater was collected adjacent to coral heads, placed on ice, and filtered on board the research vessel through a series of 25 mm diameter, 1.6 µm nominal pore-sized GF/A glass microfiber filters (Whatman International Ltd., Piscataway, NJ) and 13 mm diameter, 0.2 µm pore-sized polyethersulfone membrane filters (Supor-200; Pall Corp., East Hills, NY). Filters were then stored frozen in 250 µL of DNA lysis buffer (20 mM Tris-HCl pH 8.0, 2 mM EDTA pH 8.0, 1.2% v/v Triton X100) (Suzuki et al. 2001). All samples were transported to the Hawaii Institute of Marine Biology where they were further processed for DNA analysis.

DNA extraction and T-RFLP of bacterial SSU rRNA genes

Coral samples were thawed on ice and a flame-sterilized core borer was used to remove a 6-mm diameter, 6-mm deep core from each of the three subsamples per colony. Cores from each colony were placed into a sterile bag containing 2 mL of 0.2 µm-filtered 10X Tris EDTA (100 mM Tris, 10 mM EDTA) buffer solution (pH 7.4). An air gun outfitted with a sterile pipette tip was used to remove coral tissue from the skeleton. The coral tissue slurry was centrifuged at 19,900 RCF for 30 min at 4˚C. The supernatant was removed and the remaining sample pellet was frozen at -80˚C until further processed.

Genomic DNA was extracted from coral and seawater samples using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) according to the manufacturer's protocol. Total genomic DNA yield was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen Corp., Carlsbad, CA, USA) and SpectraMax M2 plate reader (Molecular Devices Corp., Sunnyvale, CA, USA).

Approximately 69 ±34 ng of genomic DNA was used as template for polymerase chain reaction (PCR) amplification of bacterial small subunit (SSU) ribosomal RNA (16S rRNA) genes in preparation for terminal-restriction fragment length polymorphism (T-RFLP) analysis (Liu et al. 1997).

The 50 µl PCR reactions included 1X MasterTaq reaction buffer (Eppendorf AG, Hamburg, Germany), 2.25 mM Mg2+, 0.5X TaqMaster reaction enhancer (Eppendorf), 0.2 mM each of the fluorescently labeled general bacterial SSU rRNA gene oligonucleotide primer 27F-B-FAM (5'-FAM-AGRGTTYGATYMTGGCTCAG-3') and universal SSU rRNA gene oligonucleotide primer 1492R (5'-GGYTACCTTGTTACGACTT-3'; Lane, 1991), 0.2 mM of each deoxynucleotide (Promega, Madison, WI), 2.5 units of MasterTaq DNA polymerase (Eppendorf), and sterile water. PCR reactions were carried out in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) with an initial incubation of 3 min at 95°C, followed by 30 cycles of 30 sec at 95°C, 1 min at 65°C (decreasing by 0.5°C per cycle), and 2 min at 72°C. Reactions were concluded with 10 cycles of 30 sec at 95°C, 1 min at 50°C, and 2 min at 72°C, and 1 cycle of 30 sec at 95°C, 1 min at 50°C, and 20 min at 72°C. Template-free PCR reactions were used as negative controls.

Fluorescently labeled PCR amplicons were purified using the QIAquick Multiwell PCR Purification System (Qiagen Inc., Valencia, CA) and approximately 100 ng of each purified amplicon was digested in a 10 µL reaction containing 5 units of HaeIII restriction endonuclease (Promega, Madison, WI). After a 6-h incubation at 37ºC, digests were purified via gel filtration chromatography using the Millipore MultiScreen Assay System (Millipore Corp., Billerica, MA) paired with Sephadex G-50 Superfine (GE Healthcare, Piscataway, NJ). Purified products were adjusted to a final concentration of 30 ng µL-1 and separated on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Data Processing Description

Size and relative abundance of T-RFs was determined using GeneMapper software (Applied Biosystems) and operational taxonomic units (OTUs) were defined as fragments between 50 and 600 base pairs in length. Fragment lengths were rounded to the nearest integer value, aligned, and manually checked for possible errors in peak determination. A variable percentage threshold method [C. A. Osborne, G. N. Rees, Y. Bernstein, and P. H. Janssen (2006) New threshold and confidence estimates for terminal restriction fragment length polymorphism analysis of complex bacterial communities. Applied and Environmental Microbiology, 72 (2):1270–1278] was used to normalize samples. Finally, peaks were transformed into relative abundance units by dividing integrated peak areas by the total peak area for an individual sample.

BCO-DMO Processing:

original file: Salerno_biogeography_2015_data.xlsx
- added conventional header with dataset name, PI name
- renamed parameters to BCO-DMO standard
- changed lat and lon significant digits to 4
- transposed trf rows to columns
- combined metadata with trf data
- ran rows-to-columns.pl script to transform T-RF length and abundance rows to columns
- combined coral and seawater data
- served the flat file, sorted by latitude (north to south)

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Data Files

File
coral_seawater_trf_sort.csv (Comma Separated Values (.csv), 3.72 MB) MD5:912f31c8f20534271c57d9b07a8e5efd Primary data file for dataset ID 553101

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Parameters

Parameter	Description	Units
sample_type	whether sample is from coral specimen or seawater	unitless
sample	sample identification	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
location	geographic location of sampling	unitless
year	year sampled	YYYY
site	site identification	unitless
habitat	habitat type relative to reef	unitless
depth_ft	sample depth	feet
temp	water temperature	degrees Celsius
TRF_length	terminal restriction fragment lengths	base pairs
abund	relative abundance of T-RF's	proportion

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA)
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

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_2005

Website	https://www.bco-dmo.org/deployment/553110
Platform	R/V Hi'ialakai
Start Date	2005-01-01
End Date	2006-12-31
Description	coral reef sampling

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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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