Terminal restriction fragments from 16S rRNA genes amplified from coral-associated microbes in response to temperature stress from Kaneohe Bay, Oahu, Hawaii in 2007 (MiCoDe project)

Website: https://www.bco-dmo.org/dataset/553208
Version: 2015-02-20

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

Contributors	Affiliation	Role
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Table of Contents

Dataset Description

This dataset includes the abundance of terminal restriction fragments from 16S rRNA genes amplified from coral-associated microbes. An array of closed system, precision-controlled experimental aquaria were used to investigate shifts in the bacterial communities associated with the Hawaiian reef building coral *Porites compressa* in coral fragments exposed to an abrupt 1◦C increase in seawater temperature above ambient summer levels following a 10 d acclimation at ambient temperature. Terminal restriction fragment length polymorphism analysis (T-RFLP) of bacterial small subunit ribosomal RNA (SSU rRNA) genes was conducted.

Related Reference:

Salerno, J. L., Reineman, D. R., Gates, R. D., and Rappé, M. S. (2011) The effect of a sublethal temperature elevation on the structure of bacterial communities associated with the coral *Porites compressa*. Journal of Marine Biology 2011. doi:10.1155/2011/969173

Methods & Sampling

Sample Collection and Experimental Design: On August 4, 2007, approximately 45 branch tips of *Porites compressa* (Dana 1846) 3 to 5 cm in length and 1 to 1.5 cm in diameter were removed from each of three coral colonies judged as nondiseased by gross visual assessment in central Kaneohe Bay off of the island of Oahu, Hawaii, in the Pacific Ocean. Source colonies were sampled at a depth of approximately 4m and were located within 10m of each other. Three fragments from each colony were immediately frozen in liquid nitrogen for analysis of bacterial community structure associated with the source colonies. The remaining fragments were immediately placed in containers with aerated seawater and transported back to the Hawaii Institute of Marine Biology (HIMB). In addition to the coral sampling, water samples were collected to characterize the bacterial communities in the planktonic environment surrounding the corals. Approximately 1 L of seawater was collected ~1.5m above the reef, filtered through 25mm diameter, 0.2 µm pore-sized polyethersulfone

membrane filters (Supor-200; Pall Corp., East Hills, NY), and frozen for DNA analysis in 250 µL of DNA lysis buffer (20mMTris-HCl pH 8.0, 2mM EDTA pH 8.0, 1.2% v/v Triton X100) [28].

Upon return to the laboratory at the HIMB, coral fragments were rinsed with 1 µm-filtered seawater and placed in a holding tank of 1 µm-filtered seawater maintained at the ambient temperature of 27°C. Four fragments from each colony were mounted randomly in each of eight 0.76mm thick clear vinyl sheets (Figure 1). A sheet was placed in each of eight independently controlled 60 L experimental aquaria. An additional 2-4 extra fragments per colony were also mounted in sheets as backups for fragment mortality. To minimize exposure to disturbance and handling upon removal from the reef, coral samples were collected between 0900 and 0945 and mounted in their final position in the aquaria by 1400 the same day.

All aquaria were filled with 1 µm-filtered, UV-treated seawater, which recirculated through the individual tanks for the duration of the experiment. Each tank was equipped with a full spectrum light (white and UV) on a 12:12 hr light:dark cycle, a protein skimmer, and a submersible pump flowing at 20 gal min-1. The volume of seawater in each tank was monitored daily and salinities kept constant by the addition of sterile, milli-Q freshwater. Temperature and light levels in the tanks were monitored with Onset Computer HOBO temperature and light pendant loggers (UA-002-64 Onset Computer Corporation, Bourne, MA). The loggers were fixed horizontally (light meter facing up) to the vinyl sheets containing coral fragments with cable ties, and the sheets supporting the mounted corals were fixed to the bottom of the tank with suction cups. The seawater temperature was maintained at the ambient temperature of 27°C for 10d to allow corals to acclimate.

After the acclimation period, four tanks were randomly assigned as controls (tanks 1, 8, 9, and 13) and four as treatment (tanks 3, 4, 15, and 16). At 1300 on that day, seawater and coral bacterial communities were sampled, and the seawater temperature in the treatment tanks abruptly increased to 28°C (+1° C over) in treatment tanks. Corals were subsequently sampled from the aquaria at the same time of day (i.e., initiated at 1300 and completed by 1430) after 2, 4, and 6 d of incubation and aquarium seawater after 2 and 7 d. Coral sampling consisted of removing one randomly selected coral fragment per colony per tank and freezing it in liquid nitrogen. Seawater (250 mL) was removed from each tank using acid-washed polycarbonate bottles and subsequently filtered through 25mm diameter Supor-200 membranes (Pall Corp.). Filters were placed in 200 µL of DNA lysis buffer and stored at -80°C until further processed.

T-RFLP of Bacterial SSU rRNA genes: Individual coral fragments were thawed, and a flame-sterilized stainless steel core borer was used to remove three random subsamples. Each subsample consisted of a 6-mm diameter, 6-mm deep core that included the coral tissue, overlying mucus layer, and underlying skeleton. The three subsamples were placed into one sterile bag containing 2 mL of 0.2 µm-filtered 10X Tris EDTA (100 mM Tris, 10 mM EDTA) buffer solution (pH 7.4) and airbrushed with an air gun and sterile pipette tip. The resultant slurry was centrifuged at 19,900 RCF for 30 min at 4 °C. The supernatant was subsequently removed from the sample and the remaining sample pellet was frozen at − 80 °C until processed further. After thawing, genomic DNA was extracted from the coral tissue pellets and seawater filters using the PowerSoil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, CA) according to the manufacturer's protocol. Genomic DNA was eluted in sterile, 0.1 µm-filtered water and stored frozen at − 20 °C. Total genomic DNA yield was assessed on a SpectraMax M2 plate reader (Molecular Device Corp., Sunnyvale, CA, USA) using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen Corp., Carlsbad, CA, USA), prepared according to manufacturer's protocol.

For terminal restriction fragment length polymorphism (T-RFLP) analysis, the general bacterial primers 27FB-FAM (5-FAM-AGRGTTYGATYMTGGCTCAG-3) and 1492R (5-GGYTACCTTGTTACGACTT-3) were used for the amplification of small subunit ribosomal RNA (16S rRNA) genes from each sample via the polymerase chain reaction (PCR). The MasterTaq System (Eppendorf AG, Hamburg, Germany) was used for all PCR reactions, which were composed of the following (final concentrations): 1X MasterTaq reaction buffer, 2.25 mM Mg^{2+}, 0.5X TaqMaster reaction enhancer, 0.2 mM each of the forward and reverse primers, 0.2 mM of each dNTP (Promega, Madison, WI), approximately 160 to 280 ng of genomic DNA template, 2.5 units of MasterTaq DNA polymerase, and sterile water to a final reaction volume of 50 µl. A MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA) and touchdown protocol were used, which, after a 3 min incubation at 95 °C, consisted of 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. This was followed by 10 cycles of 30 sec at 95 °C, 1min 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. The fluorescently labeled amplicons were purified using the QIAquick PCR purification kit (Qiagen Inc.) following the manufacturer's instructions. Approximately 100 ng of each purified amplicon was subsequently digested in a 10 µl reaction containing 5 units of HaeIII restriction endonuclease (Promega, Madison, WI) at 37 °C for 6 hours. After purification via gel filtration chromatography with Sephadex G-50 (Amersham Biosciences, Sweden), the restricted samples were adjusted to a final concentration of 35 ng μl^{-1} and separated via capillary electrophoresis on an automated ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Data Processing Description

GeneMapper software (Applied Biosystems) was used to estimate the size and relative abundance of T-RFs, which were defined as fragments between 42 and 613 base pairs in length after restriction as described above. Fragment lengths were rounded to the nearest integer value, aligned, and manually checked for possible errors in peak determination due to such factors as instrument variability, and so forth. The threshold below which peaks were excluded was determined via the variable percentage threshold method as described in 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.

BCO-DMO Processing:

original file: Salerno_MarBiol_2011_data.xlsx
- added conventional header with dataset name, PI name, source information
- renamed parameters to BCO-DMO standard
- sample_id's starting with a '+' were renamed with Tmt
- added lat and lon columns
- sorted by sample_id, treatment, tank, colony, time_elapsed - 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

[table of contents | back to top]

Data Files

File	
coral_2011_reformat_v2.csv	(Comma Separated Values (.csv), 2.86 MB) MD5:aa215931f27d9d4b13f17f1c909b5d67
Primary data file for dataset ID 553208	

[table of contents | back to top]

Parameters

Parameter	Description	Units
sample_type	either coral sample or seawater	unitless
treatment	Treatment= tank temperature increased abruptly by 1 degree C; Control=no change in tank temperature; Field_sample=coral or water collected in Bay; Source=seawater collected from HIMB seawater system	unitless
lat_coll	latitude of original sample collection; north is positive	decimal degrees
lon_coll	longitude of original sample collection; east is positive	decimal degrees
tank	tank number	unitless
colony	colony number	unitless
sample_id	sampe identification	unitless
time_elapsed	time elapsed since	hours
description	short description of tank; colony; etc.	unitless
TRF_length	terminal restriction fragment length	base pairs
abund	abundance of the TRF	TRF's

[table of contents | back to top]

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	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	plate reader
Generic Instrument Name	plate reader
Dataset-specific Description	SpectraMax M2 plate reader (Molecular Device Corp., Sunnyvale, CA, USA)
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.

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)

[table of contents | back to top]

Deployments

Rappe_2007

Website	https://www.bco-dmo.org/deployment/553217
Platform	Hawaii_reef
Start Date	2007-08-01
End Date	2007-08-31
Description	coral sampling for lab experiments

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]