Specificity of associations between bacteria and Pocillopora meandrina during early development in Kaneohe Bay, Oahu, Hawaii from 2008 (MiCoDe project)

Website: https://www.bco-dmo.org/dataset/553595
Version: 2015-03-26

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

Contributors	Affiliation	Role
Rappe, Michael	University of Hawaii at Manoa (HIMB)	Principal Investigator
Apprill, Amy	University of Hawaii at Manoa (HIMB)	Contact
Copley, Nancy	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Dataset Description

In this study, the specificity of bacteria associating with the coral *Pocillopora meandrina* was investigated by exposing coral embryos to various strains of cultured marine bacteria, sterile seawater, or raw seawater and examining the identity, density, and location of incorporated cells.

This dataset includes terminal restriction fragment length polymorphism analysis (T-RFLP) of small-subunit rRNA genes of *Pocillopora meandrina* exposed to *Roseobacter* and SAR11 clades of the *Alphaproteobacteria*, a *Pseudoalteromonas* species of the *Gammaproteobacteria*, and a *Synechococcus* species of the *Cyanobacteria* phylum.

Related Reference:

These data are published in Apprill, A., H. Q. Marlow, M. Q. Martindale, and M. S. Rappé (2012) The specificity of associations between bacteria and the coral *Pocillopora meandrina* during early development. Applied and Environmental Microbiology. 78:7467-7475

Methods & Sampling

Exposure of P. meandrina embryos and larvae to bacterial cultures. On 21 April 2008, fragments of healthy P. meandrina colonies were removed from the reef flat in Kaneohe Bay, Oahu, HI and held in seawater aquaria for 2 days prior to spawning on the morning of 23 April 2008. Eggs from 10 colonies were combined, fertilized with sperm for 30 min, and subsequently rinsed using seawater sterilized by the method outlined below. The embryos were placed in individual 100-mm-diameter petri dishes containing raw seawater, sterile seawater, or individual bacterial strains as treatments. The bacterial strains utilized in this study are single-strain laboratory cultures that represent some of the major groups of planktonic bacteria present in Kaneohe Bay seawater, as well as an isolate from an adult coral. Water for the sterile-seawater treatment was collected from the surface of Kaneohe Bay 4 days prior to spawning and sterilized by tangential flow filtration (TFF) using

a Millipore Pellicon 2 mini-TFF unit equipped with a 30-kDa-cutoff regenerated-cellulose filter cassette (Millipore Corp., Billerica, MA). The sterility of this water was confirmed by microscopy daily. The raw (nonsterile)-seawater treatment was prepared by coarsely filtering Kaneohe Bay surface seawater through a 1.6-um-pore-sized GF/A filter (Whatman International Ltd., Kent, United Kingdom) in order to exclude zooplankton and larger organisms. Bacterial treatments were established by growing bacterial strains in liquid batch culture and subsequently incubating diluted bacterial cells with rinsed coral planulae. After 24 h of incubation, planulae were removed, rinsed, and incubated with the same bacterial strain, freshly diluted. After repeating the treatment again at 48 h and incubating the planulae for another 24-h period (T=72 h), free-living bacterial cells were counted via microscopy (described below) and a subsample collected for community structure characterization. At 170 h, planulae were rinsed and collected for community structure characterization and microscopy analysis, and subsamples of free-living bacterial cells were again collected for characterization of bacterial community structure. This characterization was performed in order to assess whether the bacterial community was altered following incubation with coral embryos.

Axenic cultures of each strain were grown in specific media: Roseobacter strain HIMB1 and SAR11 strain HIMB4 were grown in low-nutrient medium (sterile seawater amended with ammonia and phosphate), Synechococcus strain HIMB12 was grown in low-nutrient medium amended with dilute carbon additions (D-glucose, D-ribose, pyruvate, succinate, ethanol, glycerol and N-acetylglucosamine, each at 0.001% final concentration), and Pseudoalteromonas strain HIMB1276 was grown in R2A medium with seawater as the base (Sigma-Aldrich, St. Louis MO). Cells were grown in 12-h light/dark cycles at 30°C. Every 24 h, 30-ml treatments of each bacterial strain were prepared by diluting growing cultures 10x to 1,000x (e.g., adding 1 to 0.004 ml of culture to 30 ml total) into sterile seawater.

Planulae were rinsed daily using 40-um-mesh-size cell strainers (Fisher Scientific, Pittsburg, PA) and sterile seawater and placed into new petri dishes containing fresh treatments. Treatments were initially replicated in triplicate, but after high embryo mortality during the first 24 h, replicates were subsequently combined to ensure that adequate material was available for the duration of the experiment. After 72 h (i.e., following three 24-h treatments), the abundance of microbial cells present in the treatment water exposed to the planulae was determined by microscopic enumeration as described above. Additionally, after 72 and 170 h (i.e., following three and seven 24-h treatments, respectively), subsamples of 30 to 50 ml of treatment water were filtered onto 13-mm-diameter, 0.2-_mpore- size polyethersulfone membrane filters (Supor 200; Pall Gelman, Inc., Ann Arbor, MI). Filters were stored at_80°C inDNAlysis buffer (20 mM Tris-HCl [pH 8.0], 2 mM EDTA [pH 8.0], 1.2% [vol/vol] Triton X-100) for subsequent DNA extraction. After 170 h, 100 to 200 planulae per treatment were preserved for microscopy and frozen to -20°C as described above, and 50 to 100 planulae per treatment were preserved in 250 ul of lysis buffer and frozen to -80°C for subsequent DNA extraction.

T-RFLP of bacterial SSU rRNA genes. DNA was extracted using the DNeasy tissue kit (Qiagen, Inc., Valencia, CA) with modifications and quantified using the PicoGreen fluorescent assay (Invitrogen Corp., Carlsbad, CA) on a SpectraMax M2 plate reader (Molecular Devices Corp., Sunnyvale, CA). For terminal restriction fragment length polymorphism (T-RFLP) analysis, bacterial small-subunit (SSU) rRNA genes were amplified via PCR using oligonucleotide primers 27F-B-FAM (5'-AGRGTTYGATYMTGGCTCAG-3') and 519R-VIC (5'-GWATTAC CGCGGCKGCTG-3'), with "FAM" and "VIC" indicating 5'-end labeling with FAM or VIC fluorochromes, respectively. Each 50-ul PCR mixture contained 2 U of Sahara enzyme (Bioline USA, Inc., Taunton, MA), 1x Sahara reaction buffer, 2 mM Sahara MgCl2, 200 uM each deoxynucleoside triphosphates (dNTPs), 200 nM each primer, and 100 ng of template genomic DNA (up to 1 ug for samples that did not amplify at lower concentrations). After an initial denaturation step at 95°C for 5 min, the reaction conditions were as follows: 29 cycles of 95°C denaturation for 30 s, 55°C annealing for 1 min, and 72°C extension for 2 min, concluding with an extension at 72°C for 20 min. The reactions were performed in a MyCycler personal thermal cycler (Bio-Rad Laboratories, Hercules, CA). Products were purified using the QIAquick PCR purification kit (Qiagen, Inc.) and subsequently restricted in a 10-ul reaction mixture containing 100 ng of purified amplification product, 2 ug of bovine serum albumin (BSA), 1x enzymatic reaction buffer, and 5 units of HaeIII restriction endonuclease (10 units per ul; Promega, Madison, WI) for 7 h at 37°C. Restriction digests were purified using the QIAquick nucleotide removal kit (Qiagen, Inc.).

Data Processing Description

30 ng per ul of each product was electrophoresed on an ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA). Operational taxonomic units (OTUs) were identified as individual T-RF peaks from both the forward and reverse labeled primer for fragments between 33 and 550 bp in length. To account for small differences in the amount of DNA loaded on the ABI 3100, the data were normalized by excluding peaks that contributed less than 0.05% of the total peak area for each sample.

BCO-DMO Processing:

original file: Apprill_AEM_2012_data.xlsx
- added conventional header with dataset name, PI name, source information
- renamed parameters to BCO-DMO standard
- added age (hours) of planulae to media rows
- replaced blank space with _ and removed commas
- replaced blank cells with NA
- 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

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Data Files

File
devel_2012_r2col.csv (Comma Separated Values (.csv), 364.21 KB) MD5:5e67b102896a284ab48c611344ddfc34 Primary data file for dataset ID 553595

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Parameters

Parameter	Description	Units
species	species	unitless
sample_id	sample identification	unitless
description	sample description	unitless
age	age of coral embryo	days
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
location	collection location	unitless
habitat	collection habitat	unitless
depth	depth	meters
TRF_length	terminal restriction fragment length	base pairs
abund	abundance of the TRF	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	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)

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Deployments

Rappe_2008

Website	https://www.bco-dmo.org/deployment/553607
Platform	Hawaii_reef
Start Date	2008-04-21
End Date	2008-04-21
Description	coral collection for lab studies

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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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