T-RFLP, response of the microbial community to coral spawning, lagoon and reef flats in Kaneohe Bay, Oahu, Hawaii during 2006 - 2007 (MiCoDe project)

Website: https://www.bco-dmo.org/dataset/553477
Version: 2015-03-12

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

The response of the microbial community to coral spawning was investigated over a period of 18 mo, from January 2006 to July 2007, in reef flat and lagoon environments of a subtropical embayment (Kaneohe Bay, Oahu, Hawaii, USA). The composition of the bacterioplankton community was characterized using terminal restriction fragment length polymorphism (T-RFLP) analysis of bacterial small-subunit (SSU) ribosomal RNA genes in parallel with measurements of microbial cell abundances, bacterial production via 3H-leucine incorporation, and seawater biochemical parameters. Abundance of coral eggs, zooplankton, and bacterial, and chlorophyll were measured along with nutrient analysis and physical parameters.

Related Reference:

These data are published in Apprill, A. M., and M. S. Rappé (2011) Response of the microbial community to coral spawning in lagoon and reef flat environments of Hawaii, USA. Aquatic Microbial Ecology, 62:251-266.

Methods & Sampling

T-RFLP analysis of bacterial SSU rRNA genes. Approximately 1 l of seawater was filtered through a GF/A glass microfiber membrane pre-filter (1.6 µm nominal pore size, Whatman International) followed by a 13 mm diameter, 0.2 µm pore-sized polyethersulfone membrane (Supor 200, Pall Gelman). Filters were stored at -80°C in 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). Genomic DNA was extracted from the 0.2 µm pore-sized polyethersulfone membranes using a modified version of the DNeasy Tissue kit (Qiagen) (Becker et al. 2007), and quantified using a PicoGreen fluorescent assay (Invitrogen) on a SpectraMax M2 plate reader (Molecular Devices). For terminal restriction fragment length polymorphism (T-RFLP) analysis (Liu et al. 1997), bacterioplankton small-subunit (SSU) ribosomal RNA (rRNA) genes (including those of heterotrophic bacteria, cyanobacteria, and eukaryotic plastids) were first amplified via the polymerase chain reaction (PCR) using the bacterial primers 27F-B-FAM (5'-AGRGTTYGATYM TGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG- 3'), with 'FAM' indicating 5' end-labeling of the 27F-B

primer with the 6-carboxyfluorescein (FAM) fluorochrome. Each 50 µl PCR reaction contained 0.625 U of PicoMaxx high-fidelity DNA polymerase (Stratagene), 1× PicoMaxx reaction buffer, 200 µM of each deoxynucleoside triphosphate (dNTP), 200 nM of each primer and 10 ng of environmental genomic DNA template. After an initial denaturation step at 95°C for 5 min, the reaction conditions were: 24 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). Amplification products were purified using the QIAquick PCR Purification Kit (Qiagen), and subsequently restricted in a 10 µl reaction containing 100 ng of purified amplification product, 2 µg of bovine serum albumin (BSA), 1× enzymatic reaction buffer, and 5 units of HaeIII restriction endonuclease (Promega) for 7 h at 37°C. Restriction digests were purified using the QIAquick Nucleotide Removal Kit (Qiagen), and 30 ng µl-1 of each product was subsequently electrophoresed on an ABI 3100 Genetic Analyzer (Applied Biosystems). GeneMapper software (Applied Biosystems) was used to estimate the length (in base pairs) and relative abundance of the resulting fragments. Operational taxonomic units (OTUs) were identified as terminal restriction fragments (T-RFs) detected between 33 and 550 bp in length. To account for small differences in the amount of DNA loaded on the ABI 3100, data were normalized by excluding peaks that contributed to <0.05% of the total peak area for each sample (Sait et al. 2003). An average of 2 peaks were removed from the samples, with a minimum of no peaks and a maximum of 19 peaks removed. An in-house dataset linking cloned bacterial and plastid SSU rRNA gene sequences to T-RFLP profiles from Kaneohe Bay seawater was analyzed in order to putatively identify T-RFs of interest (Yeo et al. unpubl.).

Data Processing Description

Statistical analyses. Normalized T-RFLP data were analyzed using PC-ORD software (MjM Software Design). Multi-response permutation procedure (MRPP) analysis was employed to test for significant differences in T-RFLP bacterioplankton communities between sites (McCune & Grace 2002). Species indicator analysis was used to identify T-RFs which were significantly different between parameters (Dufrene & Legendre 1997, McCune & Grace 2002). Non-metric multidimensional scaling (NMS) analysis was utilized to explore relationships between individual T-RFLP samples, and was conducted using the Sorenson (Bray- Curtis) distance measure with slow and thorough autopilot criteria (McCune & Grace 2002, Fierer & Jackson 2006). Mantel tests using Sorenson (Bray-Curtis) distance measures and Monte Carlo simulations (1000 randomized runs) were employed to test the relationship between quantitative abiotic and biotic parameters against the T-RFLP profiles.

BCO-DMO Processing:

original file: Apprill_AME_2011_data_v2.xlsx
- added conventional header with dataset name, PI name, source information
- renamed parameters to BCO-DMO standard
- replaced blank space with _, * with nd, and removed commas
- sorted by site, date, sample_id
- 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	
spawn_2011_r2col.csv	(Comma Separated Values (.csv), 5.41 MB) MD5:a881dfd986d291628624c46d53e303a4
Primary data file for dataset ID 553477	

[table of contents | back to top]

Parameters

Parameter	Description	Units
sample_id	sample identification	unitless
date_coll	date collected	yyyy-mm-dd
time_coll	time collected	HH:MM
site	site	unitless
location	locations of the sites	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
depth	depth	meters
production	carbon production	ug C L-1 hr-1
egg_abund	coral egg abundance	eggs/meter^3
zoop_biomass	zooplankton dry weight	mg/m3
TOC	total organic carbon	uM
PO4	phosphate	uM
NO3_NO2	nitrate and nitrite	uM
SiO4	silicate	uM
NO2	nitrite	uM
NH4	ammonium	uM
chl_a	chlorophyll a	(ug/L)

Synech_abund	Synechococcus abundance	cells/ml
picoeuk_abund	picoeukaryote abundance	cells/ml
nonpig_bact_abund	non-pigmented bacterioplankton	cells/ml
temp	temperature	degrees Celsius
sal	salinity	ppt
O2	dissolved oxygen	mg/L
pH	pH	unitless
turbidity	turbidity	turbidity units
tide	tide height	meters above mean low water
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	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

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_2006

Website	https://www.bco-dmo.org/deployment/553487
Platform	Hawaii_reef
Start Date	2006-01-01
End Date	2007-07-31
Description	coral sampling

[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]