Sequence data accession numbers originating from coral and coral mucus mesocosm experiments conducted at the Bermuda Institute of Ocean Sciences in 2013

Website: https://www.bco-dmo.org/dataset/652916
Data Type: experimental
Version: working
Version Date: 2016-08-01

Project
» Fundamental Coral-Microbial Associations (Coral Microbial Relationships)

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Dataset Description

Sequence data accessions from the corals and coral mucus after their time in the aquaria experiments conducted at the Bermuda Institute of Ocean Sciences (32.3705 degrees N, 64.6966 degrees W)

For other related datasets, click the project link at the top of the page.

Methods & Sampling

Colonies of *Porites astreoides* corals (5-20 cm diameter, 30-90 g wet weight) were removed from 3-9 m depth on the Bermudian reefs Hog Breaker (N 32 27.5' W 64 49.8'), an unnamed reef (N 32 26.042' W 64 49.248'), and Three Hills Shoal (N 32 41' W 64 73.3') in July 2013. Ten colonies were taken from each site in compliance with the Bermuda Institute of Ocean Sciences (BIOS) Collection and Experimental Ethics Policy and were considered Limited Impact Research and as such a collection permit was not required. The colonies were immediately placed in collection bags at depth, sealed and transported in a large collection cooler (greater than 1 h) to the laboratory. At BIOS, the colonies were allowed to acclimate to a mesocosm housing and held in aerated, outdoor fiberglass mesocosms with a flow-through seawater system fed with reef water for two (Three Hills Shoal Reef colonies) or 15 days (all other colonies) prior to the start of the experimental period.

For the experiment, the mesocosms consisted of nine, 30 L static aquaria that were set up as previously described (de Putron et al. 2010). Inshore water from 20 meters off shore and one meter in depth was used to fill the aquaria and was pumped through a BIOS flow-through sample system that consisted of a coarse mesh filter and holding tanks followed by a step filtration system of 50 um, then 5 um, to remove larger organisms including some planktonic grazers. Mesocosms were then left static for the 12-day experiment. Mesocosms were randomly arranged to consist of three control, three mucus addition, and three coral mesocosms, with artificial lights providing 61 mol quanta m-2 s-1 photosynthetically active radiation during sunlight hours consistent with the low end of known compensation ranges (3-233 mol quanta m-2 s-1 according to Mass and colleagues (2007) to minimize bleaching. Mesocosms were covered with a transparent

plastic film cover and aerated with an air wand bubbler (de Putron et al. 2010). Water was collected from the mesocosms via a syringe-siphon system using silicon tubing. Inline, combusted GF/F filters (Whatman, Maidstone, UK) were attached for samples intended for dissolved organic carbon (DOC) analysis. The reef water temperature, salinity, and conductivity in all mesocosms was monitored daily throughout the experiment using a YSI Professional Plus probe (Yellow Springs, Ohio).

Over the course of 12 days, the mesocosms were subjected to one of three conditions: coral (presence/absence of colonies), mucus additions or control (no additions). For the coral mesocosms, the experimental corals (four colonies per mesocosm, three mesocosms) were placed within the mesocosms on days 0 through 4 and removed after water sampling on day 4. Corals were removed for 48-hour until after the sampling procedure on day 6, where they were held in running seawater similarly filtered as in the mesocosms, and reintroduced back into their respective mesocosms from days 6 to 10 and removed again after the sampling procedure on day 10. *P. astreoides* colonies were not fed over the course of the 12-day experimental period. For the mucus addition mesocosms, mucus was extracted from *P. astreoides* colonies (n = 18) that were not utilized in the experiment and were maintained in a separate, flow-through seawater tank. These corals (n=18) were taken into the lab and inverted on a funnel to collect mucus for 2 h, a timeframe determined from a preliminary experiment, and the corals returned to the holding aquaria with flow through seawater. Mucus from the additional colonies was pooled and 5 ml was added to each mucus addition mesocosm on days 0.2, 2, 6 and 8. Mucus from the experimental corals (n = 12; named corals A-L from coral tanks 4, 5 and 6) was sampled for community composition prior to day 0, as well as at the end of day 4 and at the end of day 10 of the experiment. For the latter two timepoints, the corals were removed from the experimental mesocosms for 48 hours.

Seawater samples (50 ml) for cell counts and fluorescence in situ hybridization (FISH) analyses were taken daily from all mesocosms, fixed with formalin to a final concentration of 10% in the dark for 20 min, and stored at -80C. Samples were thawed and 3-5 ml were filtered onto Irgalan Black stained 25 mm, 0.2 um polycarbonate filters (Nucleopore, Whatman) under gentle vacuum (100 mmHg) and stained with 1 ml of 0, 6-diamidino-2-phenyl dihydrochloride (5 ug ml-1, DAPI, SIGMA-Aldrich, St. Louis, MO) (Porter and Feig 1980). The filters were mounted onto slides with Resolve immersion oil (high viscosity) (Resolve, Richard-Allan Scientific, Kalamazoo, MI) and stored at –20 degrees celsius. Slides were then enumerated using an AX70 epifluorescent microscope (Olympus, Tokyo, Japan) under ultraviolet excitation at 100x magnification. At least 500 cells (10 fields) were counted for picoplankton abundance.

FISH was utilized to quantify the abundance of the major picoplankton phylotypes present in the seawater and mucus, and was conducted using previously published protocols and Cy3 labeled probes (Parsons et al. 2014). The bacterial and archaeal groups quantified included the SAR11 clade (152R, 441R, 542R, 732R probes), *Alteromonas spp.* (AC137R), *Vibrio spp.* (127R), Rhodobacteracea (536R), Euryarchaeota (Eury806) and Thaumarchaeota (Cren537). Fixed seawater samples (3–5 ml) were filtered onto 25 mm, 0.2 um polycarbonate filters and stored at −20 degrees Celsius with desiccant. Quarter filters were washed in 95% ethanol and then probed according to previous protocols (Morris et al. 2002; Parsons et al. 2011, 2014). The cell abundances of the picoplankton phylotypes mentioned above were then determined using image analysis (Parsons et al. 2011, 2014). Detection of Cy3-positive cells and their ratio to DAPI-positive cells was aided by image analysis using an Olympus AX70 microscope (Olympus, Japan) equipped with a Toshiba 3CCD video camera (IK-TU40A Toshiba, Japan), a computer assisted frame grabber and appropriate dichroic filters (Morris et al. 2002; Carlson et al. 2010). Brief exposure times of 1 and 5 seconds were used for DAPI and Cy3 image channels, respectively. Cy3 images were segmented with Image Pro Plus software (Media Cybernetics, Bethesda, MD) and overlaid onto corresponding segmented DAPI images (Parsons et al. 2014). Objects with overlapping signals in both Cy3 and DAPI images were counted as probe positive. The negative control was determined similarly and subtracted from the positive probe counts to correct for autofluorescence and non-specific binding. (For **Grazing Rates** Processing, see below)

Seawater picoplankton biomass for nucleic acids was taken from all experimental mesocosms on days 0, 2, 4, 6, 8, 10 and 12 of the experiment, and from coral mucus extracted from the experimental corals on days 2, 4, and 10 and processing followed a method modified from Giovannoni and colleagues (1990, 1996). 500 ml of water or 1 ml of coral mucus was filtered through a 47 mm, 0.2 um pore filter under gentle vacuum (100 mm Hg), placed into a 4 ml cryovial and stored in 1 ml of sterile sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris.HCl) at -80 degrees celsius. For DNA extractions of the tissue, mucus and coral samples, sodium dodecyl sulfate to 1% and proteinase K to 200 ug ml-1 were added to the sample and incubated at 37 degrees celsius for 30 min and then at 55 degrees celsius for 30 min. The lysates were extracted with an equal volume of phenol:isoamylalcohol:chloroform (25:1:24) followed by two subsequent equal volumes of isoamylalcohol:chloroform (1:24). The DNA was purified by precipitation using sodium acetate (3M) and isopropanol (100%) for at least 1 hour at -20oC and centrifuged at room temperature for 30 min at 20,000 x g. The resulting pellet was washed with 80% ethanol, vortexed for 30 s and centrifuged at 16,000 x g

for 10 min. The pellet was dried and stored at -20C. (See below for **Microbial Processing** description.)

Seawater samples were analyzed for macronutrients (nitrate + nitrite, nitrite, ammonium, ortho phosphate, and silicic acid) at Oregon State University using a continuous segmented flow system consisting of a Technicon AutoAnalyzer II (SEAL Analytical) and an Alpkem RFA 300 Rapid Flow Analyzer (Alpkem) as conducted previously (Apprill and Rappé 2011). DOC was determined via high temperature combustion on a modified Shimadzu TOC-V (Shimadzu Scientific Instruments, Columbia, MD) (Carlson et al. 2010). Flow cytometry was performed on 1 ml seawater preserved to a final concentration of 4% paraformaldehyde to enumerate pigmented picoeukaryotes, Synechococcus, and non-pigmented picoplankton using methods described in Apprill and Rappé (2011). High and low DNA-containing cells were enumerated following SybrGreen staining. The ANOVA and Tukey's HSD statistics were conducted as described below.

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Data Processing Description

Grazing Rates:

We use the term 'grazing' to refer to the combination of direct ingestion by *P. astreoides* and/or grazing by coral-associated protista. Specific rates (units of d-1) were calculated for total picoplankton cells (from DAPI counts), Synechococcus (from flow cytometry), and the SAR11 clade and Rhodobacteracea (from FISH counts). Grazing rates were calculated using the standard equations of Frost (1972). The growth constant (k), or net apparent growth rate for each microbial group was calculated from each mesocosm as:

$C2 = C1ek(t2-t1)$

where C1 and C2 are the cell concentrations (cells ml-1) at t1 and t2. Grazing coefficients (g) were then calculated as the difference in k between the control and the coral addition mesocosms. Uncertainty in g was calculated using uncertainty propagation (Kline and McClintock 1953). The ANOVA and Tukey ad hoc statistics were done using the aov and tukeyHSD functioHere, wns in the stats package within the R programming language. The Honesty Significant Difference is tabled as the mean HSD value with its associated p value for significance at 95% confidence for all time points (n=3) and for the four groups – picoplankton, Synechococcus, SAR11 and Rhodobacteracea.

Microbial Processing:

The microbial composition of the DNA was assessed by targeting the V4 region of the SSU rRNA gene using modified primers, 515F and 806RB, as outlined by Apprill and colleagues (2015). Triplicate 25 ul PCR reactions were conducted per sample, and contained 1.25 U of GoTaq Flexi DNA Polymerase (Promega Cooperation, Madison, WI), 5X Colorless GoTaq Flexi Buffer, 2.5 mM MgCl2, 200 M dNTP mix, 200 nM of each barcoded primer, and 1 - 4 ng of genomic template. The reaction conditions consisted of an initial denaturation step at 95 degrees celsius for 2 min, followed by 27-34 cycles of 95 degrees celsius for 20 s, 55 degrees celsius for 15 s, and 72 degrees celsius for 5 min, concluding with an extension at 72 degrees celsius for 10 min. Reactions were carried out on a Bio-Rad thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA). Reaction products (5 ul) were screened on a 1% agarose/TBE gel using a HyperLadder 50 bp standard (generally 5 ng ul-1) (Bioline, London, UK). Samples were optimized for the lowest number of cycles that resulted in an amplified PCR product detected on a gel. The three replicate reactions were purified using the QIAquick Purification Kit (Qiagen, Valencia, CA), and quantified using the Qubit fluorescent broad range dsDNA assay (Life Technologies, Grand Island, NY).

Amplicons were pooled in equimolar ratios and shipped to the University of Illinois W.M. Keck Center for Comparative and Functional Genomics and sequenced using 2x250 bp paired-end MiSeq (Illumina, San Diego, CA), as detailed previously (Kozich et al. 2013). Sequence analyses were conducted using mothur v.3.3.3 (Schloss et al. 2009) and included assembly of the paired ends, amplicon size selection (253 bp median size) and alignment to the SSU rRNA gene. Chimera detection was also conducted via UCHIME (Edgar et al. 2011) using mothur, and chimeric sequences (7–18% of sequences per sample) were removed. Taxonomic classification of sequences was conducted with the SILVA SSU Ref database (release 119) using the k-nearest neighbor algorithm. Sequences were grouped into operational taxonomic units (OTUs) using minimum entropy decomposition (MED) (Eren et al. 2015). Statistical analysis of these data was conducted using Primer (v.7, Primer-E Ltd, Ivybridge, UK). Bray Curtis similarity data was used to create Non-metric Multidimensional Scaling (NMDS) and Principle Coordinate (PCO) analyses, ANOSIMS and PERMANOVA tests with the OTU data. Significant differences between sequence percentages were compared using two-way ANOVA with Tukey's post-hoc tests per time point using Prism 7 (GraphPad Software, Inc., La Jolla, CA). Additionally, the data were tested to determine if PCR cycle number impacted the representation of any OTUs using the BEST analysis with Primer, and did not identify any OTUs correlated with PCR cycles. Sequence data are available at NCBI's SRA under accession PRJNA 312300.

Quantitative PCR (qPCR) was used to identify betaproteobacterial and archaeal ammonia monooxygenase subunit a genes (amoA) in seawater samples from the inflow, control and coral mesocosms (n = 39), and combined coral tissue plus mucus extracts from a subset of coral colonies in the coral mesocosms (n = 6). qPCR protocols were as described previously (Santoro et al. 2010), with minor modifications. Briefly, 20 ul reactions contained 10 ul mastermix (Bio-Rad ssoAdvanced SYBR), 200 nM of each primer, 1 ul of genomic DNA template, and 8.4 ul of nuclease- free water. Samples and no template controls were analyzed in triplicate along with duplicate sets of standards containing 10 to 105 templates, on a CFX96 real-time PCR machine (Bio-Rad Laboratories). The betaproteobacterial amoA qPCR assay used the 1F/2R primer set (Rotthauwe et al. 1997). The archaeal amoA assay used the Arch-amoAF/Arch-amoAR set (Francis et al. 2005) with an additional 2 mM MgCl2. The detection limit for each assay was approximately 10 genes per reaction, corresponding to a detection limit of 2 genes ml-1 seawater. amoA detection from coral tissue samples was analyzed in undiluted and 1:10 diluted samples; detection is reported as presence/absence.

DMO Notes:

-changed column names to comply with BCO-DMO standards
-filled in blank cells with "nd"
-added NCBI links for all accession numbers
-italicized species names
-separated acquisition and analysis/processing procedures as appropriate

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Data Files

File
sequence_data_accessions.csv (Comma Separated Values (.csv), 4.82 KB) MD5:5be4e44d373f0df0b3f60f34191c9083 Primary data file for dataset ID 652916

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Parameters

Parameter	Description	Units
treatment_type	mesocosm treatment type from which accession numbers were derived	unitless
tank	tank number	unitless
timepoint_name	day during duration of 12 day experiment	days
timepoint_days	timepoint within 12 day experiment	days
accession_number	SSU rRNA gene V4 region sequences; NCBI SRA Accession	unitless

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Instruments

Dataset-specific Instrument Name	AX70 epifluorescent microscope
Generic Instrument Name	Fluorescence Microscope
Dataset-specific Description	Slides were enumerated using this microscope.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments.

Dataset-specific Instrument Name	Bio-Rad thermocycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Reactions carried out on a Bio-Rad thermocycler.
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

Apprill_2013

Website	https://www.bco-dmo.org/deployment/565728
Platform	BIOS
Start Date	2012-09-15
End Date	2016-08-31
Description	High-throughput sequencing of small subunit ribosomal RNA (SSU rRNA) genes from marine environments. Coral-bacterioplankton mesocosm experiments.

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Project Information

Fundamental Coral-Microbial Associations (Coral Microbial Relationships)

Coverage: Florida Keys, Federated States of Micronesia, Red Sea, & Bermuda

Description from NSF award abstract:
Reef-building corals are in decline worldwide due in part to climate change and other human activities, and it is becoming increasingly important to understand what aspects of coral biology are degraded by environmental stress which then leads to coral mortality. It is now widely known that corals harbor communities of bacteria and archaea that are believed to play important roles in maintaining the health of their hosts, but we lack any appreciable understanding about the identity of the microbial associates regularly residing within healthy, reef-building corals. This project asks the central question: do reef-building corals harbor fundamental or persistent microbial associates that are symbiotic within their tissues? In order to address this hypothesis, the investigator will assess the identity of the bacterial and archaeal microbes using a variety of molecular and microscopy approaches that includes the identification and localization of a widespread group of coral bacterial associates belonging to the genus Endozoicomonas. The results of this study will then be used to develop additional questions about the role of these microbial associates in nutrient cycling and how they contribute to the health and survival of corals.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1233612

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