Sequences from the coral Acropora cervicornis determined before and after bleaching at the Mote Marine Laboratory in August and September 2015

Website: https://www.bco-dmo.org/dataset/847425
Data Type: Other Field Results
Version: 1
Version Date: 2023-10-27

Project
» Collaborative Research: Tracking the interacting roles of the environment, host genotype, and a novel Rickettsiales in coral disease susceptibility (Coral Rickettsiales)

Contributors	Affiliation	Role
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Abstract
This dataset contains information about sequences of coral the Acropora cervicornis collected from host colonies (genets) at the Mote Marine Laboratory in situ coral nursery in Looe Key, Lower Florida Keys, USA in August and September of 2015. The sequence data can be found in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession number SRP267474 with the associated BioProject PRJNA639601.

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Coverage

Spatial Extent: **Lat**:24.5627 **Lon**:-81.4005
Temporal Extent: 2015-08 - 2015-09

Dataset Description

Sequence data can be found in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database under accession number SRP267474 with the associated BioProject PRJNA639601. Additional metadata related to the NCBI submission are provided in Supplemental File "MIMARKS.specimen.host-associated.5.0.xlsx".

Methods & Sampling

A total of two replicate fragments (ramets) from 16 genotypically distinct host colonies (genets) were collected from the Mote Marine Laboratory in situ coral nursery in August 2015. In September 2015, another set of two ramets from 15 of the same genets of *Acropora cervicornis* were collected from the Mote Marine Laboratory in situ coral nursery (one genotype, G20, was not available due to high mortality rates). By this time the nursery corals had been experiencing anomalously high water temperatures reaching approximately ~2° Celsius (C) above historical averages, represented by 8 degree heating weeks (DHWs) under NOAA's Coral Reef Watch products (http://www.coralreefwatch.noaa.gov). These ramets were the same individuals utilized in Muller et al. (2018) to assess changes in disease susceptibility prior to and after bleaching. Genotypes were delineated in that study via microsatellite genotyping (Baums et al., 2005). When corals appeared healthy (i.e., no visual signs of bleaching) genotype 3 was completely resistant to white band disease, and genotype 7 had only 1 out of 5 replicates succumb to disease after exposure. Genotypes 3 and 7 both were resistant to white band disease even after bleaching. All ramets hosted *Symbiodinium fitti*, the main species of *Symbiodiniaceae* found in the Mote *A. cervicornis* nursery corals (Muller et al. 2018, Parkinson et al., 2018). All corals sampled in September were visibly bleached and showed a significant reduction in photochemical efficiency using Pulse Amplitude Modulator fluorometry (see Muller et al., 2018). Upon returning to the lab, all samples were flash-frozen in liquid nitrogen and stored in at -80°C until processing. A total of two polyps were scraped from each replicate using a sterile razor blade, and total DNA was extracted from polyp tissue using the MoBio Powersoil kit.

The bacterial community dynamics of each sample was analyzed using 16S rRNA Illumina sequencing on the MiSeq platform. Amplification of the 16S rRNA gene was conducted using the 515F-806R primer set, which targets the V4 region of the 16S rRNA, with barcodes on the forward primer (Apprill et al., 2015). A polymerase chain reaction (PCR) was performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, followed by a final elongation step at 72°C for 5 minutes. PCR products were checked on a 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Angencourt Ampure XP beads (Beckman Coutler, CA, USA). Then the pooled DNA library was generated using the Illumina TruSeq DNA library preparation protocol. A PCR negative control was included in library preparation but did not produce a viable library. Paired-end sequencing was performed at MR DNA (http://www.mrdnalab.com, Shallowater, TX, USA) using a single flow cell on a MiSeq following the manufacturer's guidelines.

Known problems/issues:
Genotype 20 was not sampled after bleaching due to mortality.

Data Processing Description

Demultiplexing and barcode removal was performed using sabre (v1.0) (Copyright © Nikhil Joshi, UC Davis), during which a total of 7,499,952 reads with no barcode match were discarded from the initial pool of 15,577,446 reads. A total of 8,077,494 reads across 62 samples were subsequently processed using DADA2 (v1.16) (Callahan et al., 2016) in R (v1.1.383) (R Development Core Team, 2008). DADA2 identifies unique 16S rRNA sequences at single-nucleotide resolution (amplicon sequence variants, ASVs) using a core denoising algorithm that models substitution errors in Illumina reads. Based on quality plots, forward and reverse reads were truncated at their 3′ end at 260 and 210 base pairs, respectively. Sequences were truncated at the first position having a quality score less than or equal to 10, and reads with a total expected error of >1 or with the presence of Ns were discarded, resulting in a total of 6,178,780 reads. Amplicon sequence variants (ASVs) were inferred from unique reads and paired-end reads were subsequently merged to equal 3,089,390 reads. ASVs that did not match a target length of 292 ± 2 (77 ASVs) were discarded. Two-parent chimeras (bimeras) were removed and taxonomy was assigned at 100% sequence identity using the Silva reference database (v132) in order to preserve the high resolution of ASV data (Quast et al., 2012). The Silva taxonomic classifications for the genera MD3-55 and HIMB11 were changed to Ca. Aquarickettsia and Roseobacter, respectively, in accordance with current literature identifications (Klinges et al., 2019, Durham et al., 2014). The resulting ASV table contained 2,979 unique ASVs across 62 samples and was imported into phyloseq (v1.30.0) (McMurdie & Holmes, 2013). A total of 102 ASVs were then removed from the dataset that were annotated as mitochondrial or chloroplast sequences, corresponding to 613 and 25,100 reads, respectively. Although there were no singletons in the dataset, 2,052 unique ASVs were present in only one sample each. Next, ASVs with a

total count across the dataset in the bottom first-quartile (count = 29) were removed resulting in a total of 2,618,743 total reads, 2,133 unique ASVs, and a median sample depth of 36,970 reads.

BCO-DMO Processing Description

- Imported original file named "BCO-DMO Data_acropora_cervicornis_bleaching.xlsx" into the BCO-DMO system.
- Added a column for "collection_date" in YYYY-MM format.
- Saved the final file as "847425_v1_bleaching_sequences.csv".

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Data Files

File
847425_v1_bleaching_sequences.csv (Comma Separated Values (.csv), 33.42 KB) MD5:b21234c2ef56db9d98dd25cdb66ac331 Primary data file for dataset ID 847425, version 1.

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Supplemental Files

File
MIMARKS.specimen.host-associated.5.0.xlsx (Octet Stream, 28.28 KB) MD5:6b25598a1f4c36e3ed3d65f3e34c6e27 Supplemental file for dataset ID 847425, version 1. This is an Excel file containing the MIMARKS metadata associated with the NCBI BioSample Submission.

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Related Publications

Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137. doi:10.3354/ame01753
Methods

Baums, I., Hughes, C., & Hellberg, M. (2005). Mendelian microsatellite loci for the Caribbean coral Acropora palmata. Marine Ecology Progress Series, 288, 115–127. doi:10.3354/meps288115
Methods

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. doi:10.1038/nmeth.3869
Methods

Durham, B. P., Grote, J., Whittaker, K. A., Bender, S. J., Luo, H., Grim, S. L., Brown, J. M., Casey, J. R., Dron, A., Florez-Leiva, L., Krupke, A., Luria, C. M., Mine, A. H., Nigro, O. D., Pather, S., Talarmin, A., Wear, E. K., Weber, T. S., Wilson, J. M., … Rappé, M. S. (2014). Draft genome sequence of marine alphaproteobacterial strain HIMB11, the first cultivated representative of a unique lineage within the Roseobacter clade possessing an unusually small genome. Standards in Genomic Sciences, 9(3), 632–645. https://doi.org/10.4056/sigs.4998989
Methods

Klinges, G., Maher, R. L., Vega Thurber, R. L., & Muller, E. M. (2020). Parasitic 'Candidatus Aquarickettsia rohweri' is a marker of disease susceptibility in Acropora cervicornis but is lost during thermal stress. Environmental Microbiology, 22(12), 5341–5355. Portico. https://doi.org/10.1111/1462-2920.15245

Results

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE, 8(4), e61217. doi:10.1371/journal.pone.0061217
Software

Muller, E. M., Bartels, E., & Baums, I. B. (2018). Bleaching causes loss of disease resistance within the threatened coral species Acropora cervicornis. eLife, 7. doi:10.7554/elife.35066
https://doi.org/10.7554/eLife.35066
Methods

Parkinson, J. E., Baker, A. C., Baums, I. B., Davies, S. W., Grottoli, A. G., Kitchen, S. A., … Kenkel, C. D. (2019). Molecular tools for coral reef restoration: Beyond biomarker discovery. Conservation Letters, 13(1). doi:10.1111/conl.12687
Methods

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41(D1), D590–D596. doi:10.1093/nar/gks1219
Methods

R Core Team (2008). R: A language and environment for statistical computing. R v1.1.383. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Software

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Related Datasets

IsRelatedTo

Oregon State University. Disease-susceptible and -resistant Acropora cervicornis genotypes before and after bleaching. 2020/06. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA639601. NCBI:BioProject: PRJNA639601.

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Parameters

Parameter	Description	Units
bioproject_accession	NCBI BioProject identifier	unitless
sample_name	Sample name	unitless
SRA_run_ID	SRA run identifier	unitless
SRA_study_ID	SRA study identifier	unitless
SRA_title	Descriptive title of SRA study	unitless
library_strategy	Type of library prep ("AMPLICON")	unitless
library_source	Source of genetic material ("GENOMIC")	unitless
library_selection	Library selection method ("PCR")	unitless
library_layout	Library layout ("PAIRED")	unitless
platform	Sequencing platform ("ILLUMINA")	unitless
instrument_model	Sequencing instrument model ("Illumina MiSeq")	unitless
design	Sequencing design description	unitless
filetype	Type of file, read 1 ("fastq")	unitless
filename	Name of file, read 1	unitless
filetype2	Type of file, read 2 ("fastq")	unitless
filename2	Name of file, read 2	unitless
site	Sampling site	unitless
collection_date	Date of sampling as year and month (YYYY-MM)	unitless
lat	Sampling latitude; positive values = North	decimal degrees
lon	Sampling longitude; negative values = West	decimal degrees
Host_organism	Host from which microbiome samples were collected	unitless
genotype	Microsatellite genotype ID	unitless
bleach_status	Bleach status. Either Bleached or Apparently Healthy	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
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	Pulse Amplitude Modulator fluorometry
Generic Instrument Name	Fluorometer
Generic Instrument Description	A fluorometer or fluorimeter is a device used to measure parameters of fluorescence: its intensity and wavelength distribution of emission spectrum after excitation by a certain spectrum of light. The instrument is designed to measure the amount of stimulated electromagnetic radiation produced by pulses of electromagnetic radiation emitted into a water sample or in situ.

Dataset-specific Instrument Name	polymerase chain reaction (PCR)
Generic Instrument Name	Thermal Cycler
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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Project Information

Collaborative Research: Tracking the interacting roles of the environment, host genotype, and a novel Rickettsiales in coral disease susceptibility (Coral Rickettsiales)

Coverage: at Oregon State University and in the Florida Keys at Mote Marine Laboratory

NSF Award Abstract:
Historically one of the most abundant reef-building corals in Florida and the wider Caribbean, the staghorn coral, *Acropora cervicornis*, is now listed as critically endangered primarily because of previous and reoccurring disease events. Understanding the holistic mechanisms of disease susceptibility in this coral is a top concern of practitioners engaged in conservation and restoration. The investigators recently discovered a group of parasitic bacteria common within the microbial community of *A. cervicornis* that can reduce the growth and health of corals when reefs are exposed to nutrient polluted waters. Determining how interactions among the coral host, this parasitic microbe, and the environment are linked to disease susceptibility provides critical insight and greater success of future restoration efforts. Yet the complexity of animal microbiomes and the contextual nature of disease make it difficult to identify the specific cause of many disease outbreaks. In this project, the investigators conduct experiments to explore the interactions among different genetic strains of coral and these bacteria in various nutrient scenarios to better understand how this bacterium affects the susceptibility of staghorn coral to diseases. This project also characterizes the genomics, host range, and local and global distribution of this bacterial coral parasite to determine how its evolutionary history and physiology drive disease susceptibility in this important coral species. The project trains two postdocs, one technician, and seven students (one graduate, six undergraduates) in integrative sciences that span marine science, physiology, genetics, microbiology, omics, and statistical modeling. A research-based after school program in Florida is expanded to include microbiology and create a new program module called Microbial warriors, with a

focus on women in science. The investigators produce documentary style films and outreach materials to broadly communicate the project science and conservation efforts to local and national communities via presentations at Mote Marine Lab and the Oregon Museum of Science and Industry. This project is co-funded by the Biological Oceanography Program in the Division of Ocean Sciences and the Symbiosis, Defense, and Self-recognition Program in the Division of Integrative Organismal Systems.

The investigators recently identified a marine *Rickettsiales* bacterium that, in corals, can be stimulated to grow in the presence of elevated nitrogen and phosphorous species. Based on genomic reconstruction and phylogeography, this bacteria is classified as a novel bacterial genus, *Candidatus Aquarickettsia*, and showed that it is broadly associated with scleractinian corals worldwide. Importantly, using a model system, the endangered *Acropora cervicornis* coral, the team has also shown that the growth of this bacterium in vivo is associated with reduced host growth and increased disease susceptibility. This project aims to more completely evaluate the mechanisms behind and impacts of these inducible infections on coral physiology and host-bacterial symbiosis. The investigators conduct nutrient dosing experiments on different coral genotypes with various *Rickettsiales* abundances. Using a range of omics and microscopy techniques, the team quantifies the resulting effects on holobiont phenotypes. The investigators are also comparing the genomes of these bacteria in the different Acroporid hosts and other coral genera to evaluate facets of the bacterium's evolutionary history, as well as to identify possible mechanisms of its proliferation, virulence, and host specificity. This interdisciplinary project mechanistically links nutrients to temporal changes in host, algal symbiont, and bacterial parasite physiology and also explain why there is natural variation in these responses by exploring how host and parasite genotypes and growth dynamics combined with environmental contextuality alter holobiont phenotypes.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923836
NSF Division of Ocean Sciences (NSF OCE)	OCE-1923926

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