16S rRNA sequences for outplanted Acropora cervicornis sexual recruits collected from Mote Marine Laboratory and Looe Key National Marine Sanctuary from 2020-2022

Website: https://www.bco-dmo.org/dataset/924594
Data Type: experimental, Other Field Results
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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
A pervasive association exists between the bacterium Aquarickettsia rohweri and Caribbean Acropora, as A. rohweri dominates microbiomes of field-collected samples of this coral species. In particular, this bacterial species is highly abundant in genotypes of Acropora cervicornis susceptible to white band disease with reduced abundance in disease-resistant genotypes. A. rohweri, a member of the order Rickettsiales, is hypothesized to be an obligate symbiont dependent on the coral holobiont for nutrition and energy. Many other closely-related parasites within Rickettsiales are transmitted vertically, and A. rohweri is unlikely to persist in a free-living stage due to its limited metabolic capabilities. This bacterial parasite was therefore expected to be transmitted vertically between host generations. However, phylogenomic analyses of Acropora spp. and A. rohweri did not reveal the co-evolutionary characteristics expected of a vertically transmitted symbiont. These characteristics could be obscured, however, by horizontal transmission between hosts. The identification of A. rohweri in evolutionarily distant aquatic hosts ranging from ctenophores to sponges also strongly supports horizontal transmission of this species. To better understand the transmission dynamics of Aquarickettsia, populations of this bacteria were quantified in early life stages of A. cervicornis (gametes, planula larvae, early sexual recruits, and year-old juveniles) produced and raised in the land-based nursery at Mote Marine Laboratory in the Florida Keys. These corals were produced via controlled two-parent crosses involving six different genotypes across three annual spawning events. We found that Aquarickettsia was absent from captive-raised individuals though present in parental genotypes maintained in Mote Marine Laboratory's in situ coral nursery. In March 2021, offspring were transferred to the same in situ nursery or outplanted to reef plots either near to or far from (> 50 m) adult A. cervicornis to determine if proximity to other colonies affected parasite acquisition. Corals were sampled one week, one month, and two months post-transplantation to assess timing of Aquarickettsia infection and to examine shifts in the coral microbiome overall due to transplantation. We determined that proximity to wild or outplanted A. cervicornis influenced Aquarickettsia acquisition in outplanted conspecifics that lacked Aquarickettsia prior to outplanting. Only corals that were in close proximity to adult A. cervicornis acquired Aquarickettsia, and acquisition took between 1 and 6 months. Importantly, corals outplanted far from previously-extant outplants did not acquire Aquarickettsia by a full year after transplantation to the reef. This suggests that horizontal transmission is important in the acquisition of this putative parasite, and the parasite is likely transmitted by other A. cervicornis or reef organisms primarily associated with outplanted A. cervicornis (e.g. corallivorous snails, ciliates, bearded fireworms). We also observed that not all conspecifics from the same families acquired the parasite, even within the same site. This suggests that Aquarickettsia acquisition may require prolonged contact with a vector, or that there is an element of chance in exposure to and subsequent infection with Aquarickettsia.

Table of Contents

Coverage

Location: Mote's in situ nursery. 24.56257, -81.40009, Looe Key National Marine Sanctuary 24.54093, -81.43539 and 24.54069, -81.43652
Spatial Extent: **N**:24.56257 **E**:-81.40009 **S**:24.54069 **W**:-81.43652
Temporal Extent: 2020-10-07 - 2022-04-21

Methods & Sampling

To sample each coral, 6-8 polyps were excised from outplanted corals using bone cutters and temporarily stored in individual Whirl-Paks on ice until reaching shore. Upon shore, samples were transferred from Whirl Parks using flame-sterilized tweezers placed in a 1.5mL microcentrifuge tube containing 1mL of DNA/RNA shield (Zymo Research, R1100-250, Irvine, CA, USA). Samples were transferred to a -80°C freezer for long-term storage. In preparation for DNA extractions, the samples were removed from the -80°C freezer and thawed on ice. With flame-sterilized tweezers, half of the biomass was transferred to a Disruptor Tube (Omega Bio-Tek, Norcross, GA, USA), the other half was kept as a bioarchive and returned to -80°C. DNA from each sample was isolated utilizing the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Norcross, GA, USA) with slight modifications to the manufacturer's protocol to increase yield. DNA isolates were stored at -80°C. DNA quantity and quality was assessed utilizing a NanoDrop spectrophotometer (Thermo Fisher Scientific™, Waltham, MA, USA). Samples were submitted to MR DNA for 16S rRNA PCR amplification and sequencing (www.mrdnalab.com, Shallowater, TX, USA). 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). The 16S rRNA gene V4 variable region was amplified via a 30-cycle PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, Germantown, MD) under the following conditions: 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 10 minutes was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Samples were multiplexed using unique dual indices and were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled samples were purified using calibrated Ampure XP beads (Beckman Coutler, CA, USA). Then the pooled and purified PCR product was used to prepare an Illumina DNA library. A PCR negative control was included in library preparation but did not produce a viable library. Paired-end sequencing was performed at MR DNA on an Illumina MiSeq following the manufacturer's guidelines.

Sampling locations:

Mote's *in situ* nursery. Approximate central coordinate 24.56257, -81.40009.
Site in Looe Key National Marine Sanctuary: U-10, U-11, and U-12. Approximate central coordinate 24.54093, -81.43539.
Site in Looe Key National Marine Sanctuary: Unnamed site west of U-11. Approximate central coordinate 24.54069, -81.43652.

Data Processing Description

No processing was performed on raw reads after sequencing before submission to NCBI.

BCO-DMO Processing Description

Four submitted files (Accessions_outplant_16S.tsv, outplant_MIMARKS.survey.host-associated.6.0.xlsx, SRA_metadata_outplant.xlsx, metadata_outplant_with_ELS.txt) and one file (SraRunInfo_from_ncbi.csv)

downloaded from NCBI by the data manager with extra metadata related to the 4 submitted files were joined together.

This is the process to create the final dataset using the BCO-DMO data processor Laminar.

1) In the file Accessions_outplant_16S.tsv
The column object_status was removed since it only contained the value 'new' and is metadata used by NCBI when submitting a file and not relevant to a final dataset. The empty columns filename3, filename4, assembly, and fasta_file were removed.

2) In the file outplant_MIMARKS.survey.host-associated.6.0.xlsx
Two empty columns named sample_title and bioproject_accession were removed. The column env_broad_scale_term was added to list the NCBI term definition of [ENVO:00000150] found in the column *env_broad_scale. The column env_local_scale_term was added to list the NCBI term definition of[ENVO:01000049] found in the column *env_local_scale. The column genotype was renamed mimarks_genotype so it won't conflict with the genotype column in the file metadata_outplant_with_ELS.txt and to have an identifier of what file it came from.

3) In the file metadata_outplant_with_ELS.txt, the column genotype was renamed outplant_els_genotype so it won't conflict with the genotype column in the file outplant_MIMARKS.survey.host-associated.6.0.xlsx and to have an identifier of what file it came from.

4) In the file SRA_metadata_outplant.xlsx
The empty columns filename3, filename4, assembly, and fasta_file were removed.

5) In the file SraRunInfo_from_ncbi.csv downloaded from NCBI by the data manager
This file was downloaded using the NCBI SRA Run Selector for the BioProject PRJNA1023616 to get experiment SRX accession numbers and other metadata.

6) These 4 files are joined together along with metadata_outplant_with_ELS.txt to create the final dataset.

6a) For the first join,
the file SraRunInfo_from_ncbi.csv was joined using a full outer join with SraRunInfo_from_ncbi.csv on the column containing the BioSample accession number. The resulting joined table was named join_1.
Duplicate columns were removed.

6b) For the second join,
table join_1 was joined using a full outer join with SRA_metadata_outplant.xlsx on the column containing the Sample Name. The resulting joined table was named join_2.
Duplicate columns were removed.

6c) For the third join,
table join_2 was joined using a full outer join with outplant_MIMARKS.survey.host-associated.6.0.xlsx on the column containing the Sample Name. The resulting joined table was named join_3.
Duplicate columns were removed.

6d) For the fourth join,
table join_3 was joined using a full outer join with metadata_outplant_with_ELS.txt on the column containing the sample ID which was LibraryName in table join_3 and sample_id in file metadata_outplant_with_ELS.txt.
Duplicate columns were removed.

7) Field names were renamed to BCO-DMO naming conventions. Asterix were removed and periods were replaced with underscores.

8) The combined lat and lon field with direction was converted to individual columns lat and lon without direction. The lon column was converted to a negative value because the lon direction is West. The combined lat and lon column named lat_lon was removed.

9) The collection_date was converted from the format day-Month abbreviation-year where the day is not padded and the year is a two digit year to the format of %Y-%m-%d where the day is padded and the year is a 4 digit year.

10) The columns 'sample_id' and 'LibraryName' were compared in Excel, and the columns are the same. So deleted the column 'sample_id' and kept 'LibraryName' since the sample_id column is not the sample name in a BioSample accession and 'LibraryName' is listed as the library name in the BioSample accession metadata. The

column 'SampleName' contains the sample name associated with a BioSample accession. So 'sample_id' doesn't provide any differentiating information, and can be deleted.

11) Renamed the column mimarks_genotype to NCBI_genotype because it matched the genotype names at NCBI for each BioSample value.

12) The resulting joined table was named 924594_v1_rrna_seq_outplanted_acropora_cervicornis_sexual_recruits and it is the primary dataset file.

[table of contents | back to top]

Parameters

Parameters for this dataset have not yet been identified

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Illumina MiSeq
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	
Generic Instrument Name	bone cutter
Generic Instrument Description	A bone cutter is a surgical instrument used to cut bones or coral fragments.

Dataset-specific Instrument Name	NanoDrop spectrophotometer
Generic Instrument Name	Thermo Scientific NanoDrop spectrophotometer
Generic Instrument Description	Thermo Scientific NanoDrop spectrophotometers provide microvolume quantification and purity assessments of DNA, RNA, and protein samples. NanoDrop spectrophotometers work on the principle of ultraviolet-visible spectrum (UV-Vis) absorbance. The range consists of the NanoDrop One/OneC UV-Vis Spectrophotometers, NanoDrop Eight UV-Vis Spectrophotometer and NanoDrop Lite Plus UV Spectrophotometer.

[table of contents | back to top]

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.

[table of contents | back to top]

Funding

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

[table of contents | back to top]