Sampling and experimental metadata related to 'Candidatus' Aquarickettsia rohweri transcriptome data from host Acropora cervicornis colonies collected at Looe Key, Lower Florida Keys from Apr to Jun of 2019

Website: https://www.bco-dmo.org/dataset/928636
Data Type: experimental
Version: 1
Version Date: 2024-05-24

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

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Abstract
This dataset contains sampling and experimental metadata related to 'Candidatus' Aquarickettsia rohweri transcriptome sequences housed at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject PRJNA1048415. All host colonies (Acropora cervicornis genotype ML-50) were collected from the same location, the Mote Marine Laboratory in situ coral nursery in Looe Key, Lower Florida Keys between April and June of 2019. The Rickettsiales-like bacterial parasite, 'Candidatus' Aquarickettsia rohweri (NCBI:txid2602574) is a ubiquitous coral symbiont that is strongly linked to coral disease susceptibility in staghorn coral, and is undergoing positive selection across the Caribbean. Although ‘Ca.’ A. rohweri is a putative parasite, little is known about the activity of this bacterium in coral tissue. We performed a transcriptomic analyses of ‘Ca.’ A. rohweri populations during a 6-week nutrient exposure experiment. ‘Ca.’ A. rohweri energy scavenging genes and those potentially involved during habitat transition are significantly upregulated during enrichment. Specifically, transcripts involved in signaling, virulence, two-component systems, and nutrient import genes are elevated under higher nutrients. These data support the predicted role of ‘Ca.’ A. rohweri as a highly active nutrient-responsive A. cervicornis parasite and provide a glimpse at the mechanism of induced disease susceptibility while implicating nutrient exposure in its horizontal transmission.

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Coverage

Location: Florida Keys, USA

Spatial Extent: **N**:24.66164 **E**:-81.4005 **S**:24.5627 **W**:-81.4543
Temporal Extent: 2019-05

Dataset Description

These Ca. Aquarickettsia rohweri transcriptome data were published in Speare et al, (2023, doi: 10.1101/2023.06.08.544262) and are part of NCBI BioProject PRJNA1048415.

Closely related data also part of the same NCBI BioProject were published in Klinges et al. (2023, doi: 10.1093/femsec/fiac013) which published the experiment and accompanying data (16S, coral health status, coral growth rate) from the same samples.

Methods & Sampling

Fragments (~5cm) of *Acropora cervicornis* genotype ML-50 (Coral Sample Registry (Moura et al., 2021) accession: fa13971c-ea34-459e-2f13-7bfddbafd327) were collected from the Mote Marine Laboratory in situ coral nursery in Looe Key (24.5627,-81.4005) between April and June of 2019.

Prior to experimental manipulation, fragments were allowed to acclimate to aquarium conditions for seven days. Nutrient enrichment was performed four times a day for 42 days (six weeks). Coral fragments were sacrificed for sampling: prior to nutrient exposure (T0), and after six weeks. Using sterile bone cutters, tissue was scraped from each fragment (avoiding the apical tip) and added directly to 2 mL tubes containing 0.5ml DNA/RNA shield (Zymo Research) and Lysing Matrix A (MP Biomedicals, 0.5 g garnet matrix and one 1/4" ceramic sphere). Tubes were immediately preserved at -80°C until further processing. Total RNA was extracted from 500 µl of tissue slurry using the E.Z.N.A.® DNA/RNA Isolation Kit (Omega Bio-Tek) and then stored at -80°C until further processing. Residual DNA contamination was removed from RNA isolates using the RQ1 RNase-Free DNase (Promega). Ribosomal RNA was removed using equal parts ‘plant leaf’, ‘human/mouse’ and ‘bacteria’ Ribo-Zero kits (Illumina). RNA quality and concentration were verified by BioAnalyzer (Agilent Technologies, Santa Clara, CA) and quantitative PCR, respectively. cDNA library prep and sequencing was performed at Oregon State University’s Center for Quantitative Life Sciences (CQLS) Core Laboratories with the HiSeq 3000 platform. Three biological replicates for each treatment were sequenced (n=9).

Instruments:

Mote Marine Lab flow-through seawater raceways and 4.7L tanks: held corals during experiment
boiler and chiller: controlled aquarium temperature
Sterile bone cutters: used to collect coral tissue from each fragment
-80°C Freezer: used to keep samples at a low temp prior to processing
BioAnalyzer (Agilent Technologies, Santa Clara, CA): used to verify RNA quality and concentration
Oregon State University’s Center for Quantitative Life Sciences (CQLS) Core Laboratories with the HiSeq 3000 platform: used for sample sequencing

Organism identifiers (ScientificName, NCBI Taxonomy ID, Life Science Identifier (LSID):
Acropora cervicornis, NCBI:txid6130, urn:lsid:marinespecies.org:taxname:206989
'Candidatus' Aquarickettsia rohweri, NCBI:txid2602574, N/A

Data Processing Description

This section describes how the related sequence data (raw data deposited at NCBI) were processed and analyzed for the results publication Speare et al. (2023).

Quality scores were calculated for each sequence using FastQC and MultiQC; low-quality scores (average score <20 across 5bp) were removed. Adapters were trimmed using bbduck (BBTools User Guide); successful trimming was confirmed using FastQC/MultiQC. Forward and reverse reads were then interleaved using reformat (BBTools User Guide), mapped to the ‘Ca.’ A. rohweri genome using BowTie2, and counted using HTSeq-count. The limit of detection for each gene was one read per gene. The vegan package in R was used to perform principal coordinates analysis (PCoA) using the Bray-Curtis dissimilarity index, PerMANOVA using the Adonis function, and beta-diversity using the permutest.betadisper function. Differential expression analysis was performed through DeSeq2. Data were graphed in Graphpad Prism and edited for publication using

Inkscape 1.0.

BCO-DMO Curation Notes

* Sheet 1 of submitted file "NCBI_data_A_rohweri_transcriptomes_2024.xlsx" was imported into the BCO-DMO data system for this dataset. Metadata lines above the data table were incorporated as additional data columns.
* Data were then revised by the original data submitter to add treatment information to the data table. The revised file 928636_v1_ca-aquarickettsia-rohweri-transcriptomes_withTreatment.csv was imported into the BCO-DMO data system. This table will appear as Data File: "928636_v1_ca-aquarickettsia-rohweri-transcriptomes.csv" on this dataset page.
* Additional columns added from NCBI Biosample information for organism and host.
* Additional collection information columns added from metadata provided in email correspondence (collection year and month range description).

* Column names adjusted to conform to BCO-DMO naming conventions designed to support broad re-use by a variety of research tools and scripting languages. [Only numbers, letters, and underscores. Can not start with a number]

* Coordinates for coral nursery on Looe Key were added to metadata from information provided in email correspondence.

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Data Files

File	
928636_v1_ca-aquarickettsia-rohweri-transcriptomes.csv	(Comma Separated Values (.csv), 2.19 KB) MD5:c85ce08e938d90a639625d40dc1eb068
Primary data file for dataset ID 928636, version 1	

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Related Publications

Klinges, J. G., Patel, S. H., Duke, W. C., Muller, E. M., & Vega Thurber, R. L. (2022). Phosphate enrichment induces increased dominance of the parasite Aquarickettsia in the coral Acropora cervicornis. FEMS Microbiology Ecology, 98(2). https://doi.org/10.1093/femsec/fiac013
Methods

Moura, A., Beck, B., Duffey, R., McEachron, L., Miller, M., Moore, J., Moulding, A., & Winters, R. S. (2021). Integrating Coral Restoration Data With a Novel Coral Sample Registry. Frontiers in Marine Science, 8. https://doi.org/10.3389/fmars.2021.700172
Methods

Speare, L., Klinges, J. G., Duke, W. C., Muller, E. M., & Thurber, R. L. V. (2023). Nutrient enrichment alters gene expression in 'Ca.' Aquarickettsia rohweri, promoting parasite expansion and horizontal transmission. https://doi.org/10.1101/2023.06.08.544262
Results

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Related Datasets

IsRelatedTo

Oregon State University. (2023). Ca. Aquarickettsia rowheri transcriptome. NCBI:BioProject: PRJNA1048415 [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1048415

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Parameters

Parameter	Description	Units
Sample_name	description	unitless
BioSample_Accession	BioSample accession number in the National Center for Biotechnology Information (NCBI) BioSample database	unitless
SRA	Sample accession number in the NCBI Sequence Read Archive (SRA)	unitless
BioProject	BioProject identifier at NCBI	unitless
Data_Type	Date type description (e.g. Raw sequence reads)	unitless
Scope	Sample scope description	unitless
Organism	Organism description for the sequence accessions	unitless
Collected_Host_Organism	Host organism collected	unitless
Strain	strain (as shown with NCBI BioSample)	unitless
isolation_source	Isolation source (from the host organism)	unitless
Collection_Year	Collection year (of host)	unitless
Collection_date_note	Description of month and year range during which sampling took place	unitless
Treatment	Treatment description (see "Methods & Sampling" for full explanation of treatments).	unitless

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Instruments

Dataset-specific Instrument Name	Illumina HiSeq 3000 platform
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Oregon State University's Center for Quantitative Life Sciences (CQLS) Core Laboratories with the HiSeq 3000 platform: used for sample sequencing
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	BioAnalyzer (Agilent Technologies, Santa Clara, CA)
Generic Instrument Name	Bioanalyzer
Dataset-specific Description	Used to verify RNA quality and concentration
Generic Instrument Description	A Bioanalyzer is a laboratory instrument that provides the sizing and quantification of DNA, RNA, and proteins. One example is the Agilent Bioanalyzer 2100.

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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

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