Porites lobata growth anomaly viromes and 16S amplicon libraries analyzed in the Vega Thurber lab at Florida International University, North Miami, FL (Coral Virus project)

Website: https://www.bco-dmo.org/dataset/558384
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Project
» Effects of Viruses on Coral Fitness (Coral Virus)

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Dataset Description

This collection of 9 viral metagenomes and their associated 16S amplicon data were derived from the tissues of 1) apparently healthy *Porites lobata* corals (n=3), 2) diseased portions of *P. lobata* corals (n=3) exhibiting signs of growth anomalies, and 3) apparently healthy portions of *P. lobata* corals (n=2) exhibiting signs of growth anomalies.

Status Note [12 May 2015]: These data are stored at the MG-RAST Server http://metagenomics.anl.gov/metagenomics.cgi?page=MetagenomeSearch under ID numbers: 4489302.3, 4489297.3, 4489296.3, 4489293.3, 4489285.3, 4489243.3, 4489242.3, 4489241.3. However, at this time, these data are not publicly available. Links will be provided here once the data are available online through MG-RAST.

Methods & Sampling

16S library preparation
Nucleic acid extractions were conducted using the MOBIO UltraClean Microbial DNA Isolation Kit according to the manufacturer's protocol. Resulting DNA was amplified using barcoded 16S primers (515F/806R) (Caporaso et al., 2011; Walters et al., 2011). Amplicons were amplified in triplicate with GoTaq Flexi reagents from Promega (Madison, WI, USA) using manufacturer's protocols. Triplicate reactions were pooled and cleaned using AMPure magnetic beads from Agencourt (Brea, CA, USA). Quantification of products was conducted with an Invitrogen Qubit HS dsDNA kit (Eugene, OR, USA) and quality was determined on an Agilent Bioanalzer 2100. Equimolar ratios of the amplicons were pooled and sequenced on 454 GS FLX machine at the Center for Genome Research and Biocomputing (CGRB) at OSU.

Virome preparation
Coral tissue cores from the initial sampling (October 2009) were rinsed in viral free seawater then airbrushed into a sterile Tri-cornered beaker. The airbrushed tissue was resuspended in 40 ml of 0.02 um filtered 1X PBS, transferred to a 50 ml sterile conical tube and preserved with 2 ml reagent grade chloroform. All chloroform-persevered samples were stored at 4 degrees C and shipped overnight to the Vega Thurber lab and kept at 4 degrees C upon arrival.

Twenty-four ml of supernatant were filtered through a 1.0 um filter using a swin-lock and 10 ml luer lok syringe to remove large cells and debris. Viral particles were concentrated using CsCl density gradient ultracentrifugation as described (Vega Thurber et al., 2009). Prior to processing all the samples, one sample from each health state (healthy, healthy tissue of GA colonies, and the GA itself) was chosen to determine in which densities the virons were distributed. The presence of viral particles at each density was accessed using SYBR Gold staining (Vega Thurber et al., 2009). Viral particles were primarily distributed within the 1.35 mg ml-1 and 1.2 mg ml-1 densities. The remainder of the samples were then processed and viral particles removed from the 1.35 mg ml-1 and 1.2 mg ml-1 densities in triplicate. Each resulting sample was filtered through a 0.22 um Sterivex filter to remove bacteria. The removal of bacterial cells was confirmed using SYBR Gold staining on several samples.

Viral DNA was extracted from each sample using the protocol outlined by Vega Thurber et al. (2009). To confirm that bacterial and eukaryotic contamination was removed during filtration, we PCR amplified 16S and 18S rDNA in the extracted coral samples. Viral DNA underwent multiple displacement amplification in quadruplet using GE Health Sciences Genomphi kit (Pittsburgh, PA, USA). Approximately 2 ug of viral DNA was pyrosequenced at Engencore (University South Carolina) on a Roche 454 Titanium machine. Sequence reads first underwent preprocessing to remove sequences that had low quality scores (less than or equal to 20 average), were short (less than or equal to 100 bases), and/or were duplications using Galaxy (Goecks et al., 2010).

Data Processing Description

Sequence reads underwent several preliminary bioinformatic steps.

Viromes: SFF files were converted to FASTA/FASTQ files and de-replicated using the program GALAXY (Goecks et al., 2010). Low quality reads (that is, those of100 bp in length and/or with quality scores of Q20) were removed. To eliminate any potential non-viral sequences from the data sets, the program DeconSeq was used to identify and remove reads with nucleic acid homology (based on 60% identity and 94% similarity) to eukaryotes (mouse, fish, human and mosquito), bacteria and/or archaea (Schmieder and Edwards, 2011. The coral viromes (24 samples total, 1 plate) were barcoded and pyrosequenced on a Titanium 454 platform from Roche at EnGencore (San Francisco, CA, USA) (University of South Carolina).

These data were stored and made public at the MgRast Server http://metagenomics.anl.gov/metagenomics.cgi?page=MetagenomeSearch under ID numbers: 4489302.3, 4489297.3, 4489296.3, 4489293.3, 4489285.3, 4489243.3, 4489242.3, 4489241.3

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Parameters

Parameters for this dataset have not yet been identified

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Deployments

Vega_Thurber_Coral_Virus

Website	https://www.bco-dmo.org/deployment/558381
Platform	lab_FIU

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Project Information

Effects of Viruses on Coral Fitness (Coral Virus)

Coverage: St. Thomas, US Virgin Islands, USA; Heron Island, QLD, Australia; Orpheus Island, QLD, Australia; Kona ,Hawaii, USA; Moorea, French Polynesia.

Description from NSF Award Abstract:
Corals are important ecosystem engineers, providing habitat and nutrient recycling to tropical reefs. However, coral species richness and abundance are in decline world-wide, due in large part to anthropogenic impacts stemming from global industrialization and human population growth. Over the past several decades, global coral cover is estimated to have declined between ~20 to 60%, and approximately one-third of all known reef-building corals currently face an elevated risk of extinction. Coral disease is a major contributor to this decline of tropical reefs, and therefore, investigations into the causes of and remedies to these diseases are of critical importance. Currently little is known about viruses that infect corals. This project will address this issue.

Herpes-like viruses have been shown to be produced in coral tissues after acute episodes of stress.Viral diversity characterization, however, does not inform scientists about the effects of viral infection on coral hosts. This project will investigate whether viral infection in corals leads to disease and/or reductions in coral reproductive fitness. Specifically, this project aims to compare and contrast the relative abundance and diversity of viruses present in coral tissues during episodes of diseases, particularly, growth anomalies in Porites species and white plague disease in Montastraea species. Pyrosequencing of viral DNA will be conducted on healthy and diseased corals to: i) characterize new viral types, ii) determine whether viral types are associated with particular diseases, and iii) address the central hypothesis that viruses contribute to reduced coral fitness. Sequence analysis and functional annotation of coral viromes will determine the phylogenetic and evolutionary relationships of these viruses and identify viral mechanisms of host infection and disease. The role of viruses in host fitness will be further explored using coral fecundity and larval survivorship and settlement experiments on the model coral, Acropora millepora. Viruses will be isolated from adults, egg bundles, and larvae, in order to determine the transmission mode and ontogenic fitness effects of viral infection.

This proposal will expand the coral taxa, diseases, developmental stages, and geographic regions from which viruses have been characterized, broadening our general knowledge about the diversity of these coral parasites. The examination of viral consortia in healthy and diseased corals combined with viral inoculation experiments will then take the next step and provide scientists clues about the ecological roles that viruses play in coral reef ecosystems. This combination of high-throughput sequencing and microscopy-based methods will lead to a more comprehensive picture of the diversity and role(s) of coral viruses in holobiont fitness and disease. Lastly, insight into how viruses are transmitted will give policymakers better information about how to control viral outbreaks, including limiting the spread of infection and disease.

Recent metagenomics work has begun to uncover unique viral assemblages associated with a variety of ecosystems. To a large extent, this work has focused on phages from the open ocean and temperate coasts. This project will use similar methods to investigate viruses in tropical stony corals, a group of highly threatened organisms which provide a multitude of ecosystem services to marine organisms and local communities. The characterization of viral consortia in healthy, diseased, and different life stages of corals will provide scientists clues about the roles that viruses play in the establishment, health, and resilience of these critical ecosystem engineers.

Note: Funding for this project has transferred from award OCE-0960937 to OCE-1242064, coincident with Principal Investigator's affiliation change.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1242064
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