Montastraea annularis white plague coral 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/558392
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Project
» Effects of Viruses on Coral Fitness (Coral Virus)

Contributors	Affiliation	Role
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Dataset Description

This collection of 23 viral metagenomes and their associated 16S amplicon data were derived from seawater (n=2) and tissues of 1) apparently healthy *Montastraea annularis* corals (n=2), 2) diseased *M. annularis* corals (n=7) exhibiting signs of white plague disease, 3) bleached tissues of *M. annularis* corals exhibiting signs of white plague disease (n=7), and 4) bleached tissues of apparently healthy but bleached *M. annularis* (n=5).

Data access: These data are stored at Metavir a web server designed to annotate viral metagenomic sequences (raw reads or assembled contigs). http://metavir-meb.univ-bpclermont.fr/index.php?page=Welcome. This dataset on the published viromes, can be identified under "public projects", under the project entitled “WP reads – Soffer et al 2013.” One must "launch" each library and download the associated FASTA files individually: A=bleached from diseased samples, H=healthy bleached samples, D=diseased samples, UB=unbleached healthy samples, SW= seawater, SWD=seawater diseased.

Methods & Sampling

Sampling of the scleractinian coral, *M. annularis*, was conducted at Brewers Bay, in St. Thomas, US Virgin Islands, during a concurrent WP outbreak and bleaching event in September 2010. Collections took place over 2 days at depths of 5.5–7.6 m. Temperature at depth was 29.0 degrees C. All *M. annularis* colonies used in this study were located within ~75 m of each other. Coral samples were collected using SCUBA, where two to three cores of tissue attached to skeleton were removed from each *M. annularis* colony using a 2 cm diameter corer and hammer (USVI Department of Planning and Natural Resources permit #STT-050-10). Samples were collected at depths of 5.5–7.6 m and at temperatures 29 degrees C. Colonies were within 5–7 m of each other and 75 m from shore.

Viromes: Phage and eukaryovirus particles were isolated and sequenced separately from bacterial cells (using CsCl density gradient ultracentrifugation) before sequencing (Vega Thurber et al., 2009; more details in Soffer et al., 2014). DNA was then extracted with a phenol-chloroform extraction protocol (Vega Thurber et al., 2009; Soffer et al., 2014) and amplified using non-specific MDA according to manufacturer’s protocol (GenomPhi, GE Healthcare, Pittsburgh, PA, USA). The coral virome libraries (21 coral samples) were barcoded and pyrosequenced at EnGencore (University of South Carolina) on a Roche Titanium 454 platform. The final

numbers of replicate libraries for each coral health state were: H (n = 2), B (n = 5), BD (n = 7) and D (n = 7).

Bacterial Amplicons: 500 ul of ethanol/tissue slurry was pipetted for DNA extractions. A modified organic extraction protocol was used to purify DNA (for details see Soffer, Zaneveld, and Vega Thurber (2015) Phage–bacteria network analysis and its implication for the understanding of coral disease. *Environmental Microbiology* 17:1203-1218). Isolated nucleic acids were amplified using barcoded primers 515F and 806R, which were chosen due to their ability to amplify both bacteria and archaea (Caporaso et al., 2011; Walters et al., 2011). Triplicate amplicon libraries were prepared using GoTaq Flexi reagents from Promega (Madison, WI, USA) using standard protocols and the following PCR cycle: 1 cycle of 94 degrees C for 3 min, 35 cycles of 94 degrees C for 45 s, 50 degrees C for 60 s, and 72 degrees C for 90 s, and then 1 cycle of 72 degrees C for 10 minutes. PCR products were run on a 1.5 agarose gel, triplicates pooled, cleaned using AMPure magnetic beads from Agencourt (Brea, CA, USA) and quantified with a Qubit HS dsDNA kit (Invitrogen,Eugene, OR, USA) into equimolar ratios. Quality of amplicons were determine on an Agilent Bioanalyser 2100 before being pyrosequenced on a 454 GS Junior Roche at the Oregon State University Center for Genome Research and Biocomputing Core Laboratories.

Related Publications:
Soffer,N., Brandt, M.E., Correa, A.M., Smith, T.B. and Vega Thurber, R.L. 2014. Potential role of viruses in white plague coral disease. ISME J., 8(2): 271-283. doi:10.1038/ismej.2013.137

Soffer, N., Zaneveld, J., and Vega Thurber, R. 2015. Phage–bacteria network analysis and its implication for the understanding of coral disease. Environmental Microbiology, 17:1203-1218. doi:10.1111/1462-2920.12553

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, thoseo100 bp in length and/or with quality scoresoQ20) 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 at Metavir a web server designed to annotate viral metagenomic sequences (raw reads or assembled contigs). http://metavir-meb.univ-bpclermont.fr/index.php?page=Welcome. This dataset on the published viromes, can be identified under "public projects", under the project entitled “WP reads – Soffer et al 2013.” One must “launch” each library and download the associated FASTA files individually: A=bleached from diseased samples, H=healthy bleached samples, D=diseased samples, UB=unbleached healthy samples, SW= seawater, SWD=seawater diseased.

Bacterial amplicons: 16S rRNA gene sequence libraries were analysed in QIIME and PRIMER 6 (Clarke and Gorley, 2006; Caporaso et al., 2010). First, reads were de-multiplexed based on their error correcting barcodes and filtered for quality using default parameters (quality score greater than or eaqual to 25, min length = 200, max length = 1000, and no ambiguous bases and mismatches allowed). Next OTUs were assigned at a 97% similarity threshold UCLUST (Edgar, 2010), and OTU tables constructed from the assignments. Lastly, taxonomic annotations were made to the RDP database using the RDP classifier (Wang et al., 2007). OTUs identified as chloroplasts were removed from the analysis (mean 23% +/- 0.2). Amplicon libraries were paired with all virome samples with the exception that there was an additional bleached sample was processed for bacterial analysis.

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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
NSF Division of Ocean Sciences (NSF OCE)	OCE-0960937

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