Accession numbers for Gorgonia ventalina (sea fan) sequencing data from samples collected in the Florida Keys and Puerto Rico from 2006-2010 (Climate_CoralDisease project)

Website: https://www.bco-dmo.org/dataset/3717
Version: 12 Sept 2012
Version Date: 2012-09-12

Project
» Influence of Temperature and Acidification on the Dynamics of Coral Co-Infection and Resistance (Climate_CoralDisease)

Contributors	Affiliation	Role
Harvell, Drew	Cornell University (Cornell)	Principal Investigator
Mydlarz, Laura	University of Texas at Arlington (UT Arlington)	Co-Principal Investigator
Burge, Colleen	Cornell University (Cornell)	Contact
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Table of Contents

Dataset Description

Accession numbers to NCBI's Gene Expression Omnibus (GEO) are provided for the *Gorgonia ventalina* Illumina sequences collected from reefs off the coast of Puerto Rico.

References:

Burge CA, Mouchka, ME, Harvell, CD & S Roberts. (In review) Immune response of the Caribbean sea fan, Gorgonia ventalina exposed to an Aplanochytrium parasite as revealed by transcriptome sequencing.

Methods & Sampling

Sea fan collection and husbandry
Twelve *G. ventalina* individuals were collected at Laurel Patch Reef, La Parguera, Puerto Rico in May 2010. Sea fans were cut into two, 6x9 cm pieces, and suspended in situ to heal on the reef for three days. Sea fans were then collected from the reef and moved into static 38 L aquariums at the University of Puerto Rico, Isla Magueyes Laboratories in La Parguera, PR. Fans were distributed equally, in a clonally replicated design, between six aquariums (n=4 sea fans per aquarium with sea fan specimens 1-4, 5-8, 9-12 held in individual aquariums in duplicate). Sea fans were acclimated for two days and water was changed twice daily.

Experimental Inoculations
One piece of each sea fan (n=12) was injected three times with a solution of a 5 day culture of an *Aplanochytrium* previously isolated from a sea fan. Complementary clonal pieces (n=12) were injected with QPX media only to serve as a control. After 24 hours, an area including each of the three injection points was collected from each sea fan and flash frozen in liquid nitrogen and subsequently stored at -80 C. Sea fan

samples were shipped on dry ice overnight to Cornell University and stored at -80 C until sample preparation.

Sample preparation
Each individual sea fan sample was ground in liquid nitrogen using a mortar and pestle, and the resulting powder was placed in a 2.0 mL microcentrifuge tube. Total RNA was extracted using a modified Trizol/Qiagen RNeasy protocol, whereby following the ethanol precipitation step of the Trizol manufacturer's instructions (Invitrogen, The Life Technologies CorporationTM, Grand Island, NY), the aqueous solution was added to an RNeasy column, and the Qiagen RNeasy manufacturer's instructions were subsequently followed (Qiagen, Valencia, California). DNA was removed from extracted RNA using the Turbo DNA-free treatment according to the manufacturer's instructions (Ambion Inc, The Life Technologies CorporationTM, Grand Island, NY). Removal of DNA was confirmed by using RNA (1 µl) as template in a PCR reaction targeting 18s ribosomal DNA as previously described. RNA concentrations were quantified using the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE).

Sequencing and Analysis
For each treatment, samples were pooled (n=12) using 900 ng of total RNA from each sea fan. RNA quality was assessed using an Agilent BioAnalyzer 2100 at the Cornell University Life Sciences Core Laboratory Center (CLC). Library preparation was done at the Cornell Microarray facility using the mRNA-Seq 8-Sample Prep Kit (Illumina, San Diego, California) followed by sequencing preparation using the standard cluster generation kit and 36 cycle Illumnia sequencing kit (Illumina, San Diego, California) at the Cornell University CLC. Each library was sequenced in its own individual lane where 86 bp reads were captured using an Illumina/Solexa Genome Analyzer at the Cornell University CLC. The data have been deposited in NCBI's Gene Expression Omnibus.

Data Processing Description

Data Processing:
Initially, all sequences were trimmed based on quality scores of 0.05 and the number of ambiguous nucleotides (>2 on ends). Sequences smaller than 20 bp were also removed. De novo assembly was carried out using CLC Genomics Workbench v4.0 (CLC Bio) with the following parameters: similarity=0.90, length fraction=0.8, insertion cost=3, deletion cost=3, mismatch cost=2 and minimum size = 400.

Consensus sequences (or contigs) were compared to the UniProtKB/Swiss-Prot database. Comparisons were made using the BLASTx algorithm with a maximum of 1E-6 e-value threshold.

RNA-Seq analysis was performed to determine differential gene expression patterns between Aplanochytrium exposed and control libraries. CLC Genomics Workbench v4.0 (CLC Bio) was used to map the reads to the assembled transcriptome and to obtain raw counts of sequencing reads using following parameters: unspecific match limit = 5, maximum number of mismatches = 2, minimum number of reads = 10. Statistical comparisons of count data between the control and Aplanochytrium exposed libraries was carried out using DESeq. Genes were considered differentially expressed in a given library when the adjusted p-value was less than or equal to 0.05.

To identify enriched biological themes and GO terms, the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 was used. Specifically, corresponding Swiss-Prot accession numbers for differentially expressed genes were used as the gene list and corresponding Swiss-Prot accession numbers for the assembled transcriptome was used as the background. Biological Process terms were considered significantly enriched when the p-value was less than 0.05.

[table of contents | back to top]

Data Files

File
sea_fan_sequences.csv (Comma Separated Values (.csv), 353 bytes) MD5:e54be7bd722321f35f52b67e7f1fb686 Primary data file for dataset ID 3717

[table of contents | back to top]

Parameters

Parameter	Description	Units
treatment	Exposure type: Aplanochytrium exposed = sea fan injected with a solution of Aplanochytrium culture. Control = sea fan injected with QPX media as a control.	text
accession_number	Assigned accession number and link to NCBI GEO.	unitless
site	Name of the reef where G. ventalina was collected.	text
lat	Latitude of the collection site. North = positive.	decimal degrees
lon	Longitude of the collection site. West = negative.	decimal degrees

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Automated DNA Sequencer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Each library was sequenced using an Illumina/Solexa Genome Analyzer at the Cornell University Core Laboratory Center.
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.

[table of contents | back to top]

Deployments

Coral_Dive_HM

Website	https://www.bco-dmo.org/deployment/58855
Platform	shoreside PR_Keys_Reef
Start Date	2006-09-01
End Date	2010-09-01
Description	Four dive sites for the Harvell/Mydlarz project 'Influence of Temperature and Acidification on the Dynamics of Coral Co-Infection and Resistance': Big Pine Ledges, Florida Keys: 24° 33.207 N, 81° 22.731 W Laurel patch reef, La Parguera, Puerto Rico: 17° 56.608 N, 67° 03.208 W Media Luna, La Parguera, Puerto Rico: 17°56.093 N, 67°02.931 W (3 to 18 m depths) Buoy, La Parguera, Puerto Rico: 17° 53.38 N, 66° 59.09 W (18 to 25 m depths)

[table of contents | back to top]

Project Information

Influence of Temperature and Acidification on the Dynamics of Coral Co-Infection and Resistance (Climate_CoralDisease)

Coverage: Florida Keys & Puerto Rico

This award is funded under the American Recovery and Reinvestment Act of 2009 (Public Law 111-5).

Coral reef ecosystems are highly endangered by recent increases in temperature and by projected increases in ocean acidification. Although temperature has been identified as a driver of some coral disease outbreaks, nothing is known about direct effects of acidification on host immunity and pathogen virulence, or the potential for synergism with temperature. Natural coral populations often suffer from simultaneous infection by multiple pathogens that can also influence host immune responses, but co-infection dynamics have not been investigated in invertebrate systems lacking classical adaptive immunity. Changing climate will very likely influence the outcome of single and co-infection.

This project will investigate the influence of environmental stress on co-infection dynamics of the sea fan coral, *Gorgonia ventalina*, with a fungal pathogen, *Aspergillus sydowii* and a protist parasite, SPX. The goal is to identify the mechanisms through which multiple infections, temperature and acidification modify host resistance, leading to changes in within- and among-colony rates of disease spread.

The objectives of this project are to:
(1) Identify incidence and co-infection frequency of *Aspergillus sydowii* and SPX. Detailed field surveys of the two diseases will test the hypothesis that co-infection is significant, provide valuable information about drivers of aspergillosis, and will help to characterize an emerging new sea fan disease.
(2) Investigate how co-infection influences sea fan susceptibility, resistance, and within host disease dynamics. Through manipulative lab inoculation experiments we will test the hypothesis that single infections increase susceptibility to a second pathogen.
(3) Examine the effects of temperature increase and ocean acidification on pathogen virulence, on underlying host resistance, and on the dynamics of single and co-infections.

The hypotheses that acidification will increase pathogen virulence and host susceptibility will be tested in a temperature and pH controlled experimental system. This system will also allow the potential synergistic effects of temperature and acidification on host immunity and co-infection dynamics to be explored. The primary intellectual merit of the proposed work will be a greater understanding of how changing climate mediates co-infection and immunity in a non-model invertebrate. While fungal pathogens are primarily opportunistic, labyrinthulid protozoans are recognized as primary pathogens in shellfish. Even in shellfish, little is known about co-infections involving labyrinthulids, and these protists are entirely unstudied in corals.

Publications associated with this project:
Burge CA, Douglas N, Conti-Jerpe I, Weil E, Roberts S, Friedman CS & CD Harvell. (May 2012) Friend or foe: the association of *Labyrinthulomycetes* with the Caribbean sea fan, *Gorgonia ventalina*. Dis Aquat Org. 101:1-12. doi: 10.3354/dao02487

Burge CA, Mouchka, ME, Harvell, CD & S Roberts. (In review) Immune response of the Caribbean sea fan, *Gorgonia ventalina* exposed to an *Aplanochytrium* parasite as revealed by transcriptome sequencing.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0849776

[table of contents | back to top]