Antibacterial assays of Montipora capitata mucus collected in Kaneohe Bay, Oahu, Hawaii during 2014

Website: https://www.bco-dmo.org/dataset/544898
Data Type: experimental
Version: 2014-08-28

Project
» Host-environment-pathogen interactions in a model coral disease system (coral-pathogen interaction)

Contributors	Affiliation	Role
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Dataset Description

Data access is restricted. Please contact the PI for further information.

Related datasets:

Montipora antibacterial-aqueous
Montipora antibacterial-organic
Montipora chemical fingerprints
MWS accession numbers
MWS lesion progression

Related references:

Deborah J. Gochfeld, Greta S. Aeby. 2008.Antibacterial chemical defenses in Hawaiian corals provide possible protection from disease. Mar Ecol Prog Ser 362: 119-128. doi: 10.3354/meps07418

Methods & Sampling

Mucus sampling: Mucus was obtained from the surfaces of replicate orange and red colonies of Montipora capitata collected in Kaneohe Bay, Oahu, using a sterile pipettor and frozen immediately.

Bacterial strains tested: The strains used for the antibacterial assays were selected as model systems to represent a range of potential bacterial pathogens from the marine environment, including known coral pathogens (Aurantimonas coralicida, Serratia marcescens, Vibrio coraliilyticus, Vibrio shiloi) and human enteric bacteria that have the potential to enter near-shore waters and can survive in the marine environment (Yersinia enterocolitica), and strains previously isolated from the surfaces of Hawaiian corals (Klebsiella pneumonia, Vibrio agarivorans) (Gochfeld & Aeby 2008). In addition, bacterial strains isolated from surfaces of Montipora capitata (OCN001, OCN002, OCN003, OCN008, O1-1, O2-8, R1-13, R5-5, R5-29) by Dr. Sean Callahan's lab at University of Hawaii were also used. Two of these, OCN002 and OCN008, are pathogens associated with Montipora White Syndrome (Ushijima et al. 2012, 2014).

Growth inhibition assay (Gochfeld & Aeby 2008): Assays were run in triplicate in 96 well plates. Plates contained 3 wells of bacteria + each mucus sample (n = 5 replicate corals of 2 color morphs), along with the following controls: bacteria only, media only, and mucus solution to control for the coloration of extracts. Twenty-four hour bacterial cultures in exponential growth were diluted to optical density (OD) 600, and 100 µl were added to each well. 10 µl of mucus were added to the experimental wells. Wells were mixed by carefully pipetting their contents, and an initial reading (time 0) of OD was made on a BioTek Synergy™ HT Multi-Detection Microplate Reader. Plates were then covered with foil and incubated on a shaker for 24 h and a final reading was made. Prior to each reading, wells were mixed again to resuspend any settled material.

Data Processing Description

To compare the growth curves from assays performed on different plates and at different times, we controlled for natural bacterial growth in each assay by subtracting the mean optical density of the 3 control wells (media + bacteria) from the optical density of each well containing a mucus sample in that assay. In addition, the mean optical density of the 2 wells containing each mucus solution was also subtracted from those wells to control for any color of the mucus.

BCO-DMO Processing:
added conventional header with dataset name, PI name, version date, reference information
renamed parameters to BCO-DMO standard
replaced blanks and / with underscores

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Data Files

File
assays.csv (Comma Separated Values (.csv), 1.55 KB) MD5:59e0bd37d2fe80231f2427c2417cac5f Primary data file for dataset ID 544898

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Parameters

Parameter	Description	Units
assay	coral extract material examined	unitless
color_morph	color morph of coral	unitless
coral	coral fragment identification	unitless
Aurantimonas_coralicida	mean zone of inhibition against Aurantimonas coralicida	millimeters
Klebsiella_pneumoniae	mean zone of inhibition against Klebsiella pneumoniae	millimeters
Pseudomonas_nautica	mean zone of inhibition against Pseudomonas nautica	millimeters
Serratia_marcescens	mean zone of inhibition against Serratia marcescens	millimeters
Vibrio_agarivorans	mean zone of inhibition against Vibrio agarivorans	millimeters
Vibrio_corallyliticus	mean zone of inhibition against Vibrio corallyliticus	millimeters
Vibrio_shiloi	mean zone of inhibition against Vibrio shiloi	millimeters
Yersinia_enterocolitica	mean zone of inhibition against Yersinia enterocolitica	millimeters
OC_N001	mean zone of inhibition against OC-N001	millimeters
OC_N002	mean zone of inhibition against OC-N002	millimeters
OC_N003	mean zone of inhibition against OC-N003	millimeters
OC_N008	mean zone of inhibition against OC-N008	millimeters
O1_1	mean zone of inhibition against O1-1	millimeters
O2_8	mean zone of inhibition against O2-8	millimeters
O2_12	mean zone of inhibition against O2-12	millimeters
R1_13	mean zone of inhibition against R1-13	millimeters
R5_5	mean zone of inhibition against R5-5	millimeters
R5_13	mean zone of inhibition against R5-13	millimeters
R5_29	mean zone of inhibition against R5-29	millimeters

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Instruments

Dataset-specific Instrument Name	plate reader
Generic Instrument Name	plate reader
Dataset-specific Description	BioTek Synergy™ HT Multi-Detection Microplate Reader
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

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Deployments

Aeby_2014

Website	https://www.bco-dmo.org/deployment/544868
Platform	Hawaii_reef
Start Date	2010-06-01
End Date	2014-05-31
Description	Coral reef pathogen studies.

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Project Information

Host-environment-pathogen interactions in a model coral disease system (coral-pathogen interaction)

Coverage: Kaneohe Bay, Oahu, Hawaii (21 26' N, 157 47' W)

Extracted from the NSF award abstract:

Diseases of marine organisms have emerged as a serious problem contributing to the decline of coral reef resources worldwide. Loss of coral reef habitats carry social and economic implications especially in island states, such as Hawaii, which depend on reefs for food, shoreline protection and tourism. Our ability to manage coral diseases is hampered by a lack of knowledge of which environmental variables affect disease, mechanisms of host defense, and the etiology of most of the numerous described coral diseases. The PIs of this project discovered a coral disease system that can be used as a model to explore many components of

the host-environment-pathogen triangle of disease causation. Montipora white syndrome (MWS) is an infectious disease that results in progressive tissue loss on colonies of Montipora capitata, and has been found on reefs throughout the Hawaiian archipelago. It is particularly prevalent in Kaneohe Bay, Oahu, which has a long history of reduced water quality, and this suboptimal environment sets the stage where host-pathogen interactions occur. In Kaneohe Bay, M. capitata is a major reef-building species, and is found in two color morphs (red and orange) that harbor different clades of zooxanthellae. During preliminary surveys, the PIs discovered intraspecific variability in response to MWS between color morphs. Although the red morph was dominant within survey transects (80% of the colonies), the orange morph was disproportionately affected by MWS (70% of the affected colonies). Microbial studies found a shift in bacterial communities on MWS-affected and healthy M. capitata and allowed identification of potential pathogens. Numerous bacterial strains were cultured and screened for pathogenicity and three strains, which produced lesions, were identified as potential pathogens. Two of the putative pathogens (Vibrio spp.) produced diffuse tissue whereas the other bacterial strain (Pseudoalteromonas sp.) produced acute tissue loss.

In the field, the PIs also observed two patterns of tissue loss on M. capitata; a slow, chronic pattern of tissue loss, which they followed through time with tagged colonies (chronic MWS), but also a rapid onset of acute tissue loss (acute MWS). Thus they discovered an infectious coral disease that results in significant coral mortality that has the unique component of differences in disease susceptibility among color morphs. The PIs identified three potential bacterial pathogens that will be used to investigate underlying factors affecting the coral-environment-pathogen triad of disease causation. The Hawaii Institute of Marine Biology (HIMB) is located within Kaneohe Bay allowing year-round access to reefs for research on Montipora white syndrome. The goal of this project is to investigate the host- environment-pathogen triangle of disease causation for Montipora white syndrome. The objectives of this research will be to: 1) investigate mechanisms contributing to differential disease resistance in red (less susceptible) vs. orange (more susceptible) morphs of M. capitata. The PIs will compare antimicrobial activity in the holobiont, mucus and mucus-associated bacteria of the two color morphs of M. capitata, and will compare the natural coral-associated microbial flora between the two color morphs; 2) use manipulative aquarium studies to determine whether environmental stressors (elevated temperature, nutrient stress) differentially affect the progression or transmission efficiency of MWS in red vs. orange morphs of M. capitata; 3) use challenge experiments to confirm the role of bacterial pathogens as causative agents of MWS, and to determine the response of red vs. orange morphs of M. capitata to three putative pathogens. This project will involve a multidisciplinary team to provide a broader perspective of coral disease processes. This will be the first comprehensive study conducted on a coral disease in Hawaii.

Related Publications:

Ushijima, B, Videau, P, Burger, A, Shore-Maggio, A, Runyon, C, Sudek, M, Aeby, G and S. Callahan. 2014. Vibrio coralliilyticus strain OCN008 is an etiological agent of acute Montipora white syndrome. Applied & Environ Microbiology doi:10.1128/AEM.03463-13.

Ushijima B, Videau P, Aeby GS, Callahan SM. 2013. Draft Genome Sequence of Vibrio coralliilyticus Strain OCN008, Isolated from Kane'ohe Bay, Hawai'i. Genome Announc. 2013 Oct 3;1(5). doi:pii: e00786-13. 10.1128/genomeA.00786-13. PMID: 24092784

Ushijima B, Smith A, Aeby GS, Callahan SM (2012) Vibrio owensii Induces the Tissue Loss Disease Montipora White Syndrome in the Hawaiian Reef Coral Montipora capitata. PLoS ONE 7: e46717.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0961384

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