Reduced virulence of black band disease on *Orbicella faveolata* under ocean acidification conditions.

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Introduction

One of the most well-known and widespread coral diseases is black band disease (BBD, Fig 1)[1]. BBD is known to affect at least 42 species of hard corals and gorgonians, which includes major reef-building corals[2]. BBD progresses across the infected coral colony lysing coral tissue and leaving behind exposed skeleton. The disease is characterized by a pathogenic microbial consortium that includes an oxygenic photosynthetic cyanobacterium, sulfate reducing bacteria such as *Desulfovibrio* spp., and the sulfide-oxidizing bacterium

Figure 1. Black band disease moving along a hard coral, lysing tissue and leaving behind skeleton.

The IPCC predicts the oceans temperature will continue to rise and that oceanic pH will decrease[4]. This changing environment may place stress on corals and stressed corals suffer greater mortality[5]. This may suggest that ocean acidification will allow greater rates of BBD progression and infection in coral colonies. A preliminary study conducted in July 2013 suggested that BBD virulence is negatively affected by low pH in corals but benefits from it in *ex-situ* marine broth. Three species of coral were used in the experiment, *Monstraea cavernosa, Oribcella faveolata and Pseudodiploria clivosa*, at the Mote Tropical Research Laboratory. The water used at the Tropical Research Laboratory was drawn from a well and differs in composition when compared to reef seawater, warranting a confirmation trial at Mote Marine Laboratory.

Objectives

1. Reproduce the results of the July 2013 study using oceanic reef water versus well water to assess the progression of black band disease under different pH conditions *in-situ*.
2. Quantify the bacterial community of the black band disease consortium *in-situ* and *ex-situ* under different temperature and pH regimes.
3. Quantify the bacterial community with in the coral host under different temperature and pH regimes.

Hypotheses

1. Similar to the 2013 experiment, low pH will reduce the virulence of BBD.
2. The bacterial community of the BBD in situ and ex situ will differ based on treatment.
3. The bacterial community of the coral host will differ based on treatment.

Methods

Microbial analysis of July 2013 study:
Four treatment conditions in seawater and marine broth: high pH (8.1) and high temperature (30ºC), high pH (8.1) and low temperature (26ºC), low pH (7.6) and high temperature (30ºC), low pH (7.6) and low temperature 26ºC). Samples of coral mucus and black band disease were taken from each coral and DNA isolation was performed using MoBio Power Soil kit. A subset of 16 marine broth treatments (4 of each treatment) and a subset of 36 for both mucus and black band from the corals (3 of each treatment for each species) were analyzed using 454 pyrosequencing using 16S rRNA gene primers for bacteria (3000 reads). The results were analyzed using ANOSIM and SIMPER tests on the statistical program "R"

Methods

***Orbicella faveolata* confirmation at Mote Marine Laboratory:**
Sixteen 10 x 10 cm fragments were collected from the NOAA coral nursery in the Florida Keys. The black band disease sample was collected on Looe Key reef in the Florida Keys. There were eight replicates in seawater at pH 8.1 and eight replicates in seawater at pH 7.7 (for CO2 data reference Fitzpatrick and Hall poster). Temperature was kept constant. Each fragment was infected using .03 grams of black band disease and photos were taken daily to determine rates of progression using ImageJ software (Fig 2). The surface area data were analyzed using an analysis of variance test after a rank transformation.

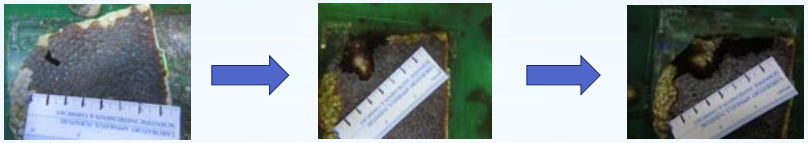

Figure 2. Coral fragment 1 (pH 7.7) on day 1, day 12 and day 19.

Results

Confirmation experiment:
There was asignificant difference in the average surface area of black band disease between the two different pH regimes (p-value = 0.0011) in the OAsys confirmation experiment. (Figure 3.)

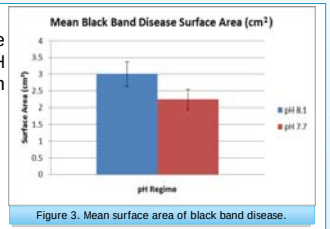

Figure 3. Mean surface area of black band disease.

ANOSIM analysis:
There were no significant differences in the microbial community between pH (fig. 4) and temperature (fig. 5) treatments n the black band disease grown in marine broth (pH p-value = 0.785, temperature p-value = 0.436). However both ordination plots show defined separation of treatments.

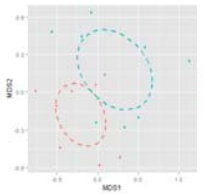

Figure 4. Ordination plot of bacterial communities from marine broth grown in high and low pH treatments.

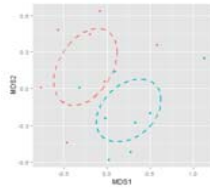

Figure 5. Ordination plot of bacterial communities from marine broth grown in high and low temperature treatments.

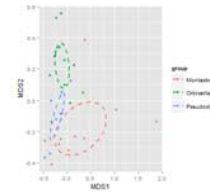

Figure 6. Ordination plot of bacterial community within black band composition among species.

There were no significant differences in microbial communities between pH (*M. cavernosa* p-value =0.423, *O. faveolata* p-value = 0.077, *P. clivosa* p-value = 0.79) and temperature (*M. cavernosa* p-value =0.223, *O. faveolata* p-value = 0. 327, *P. clivosa* p-value = 0.48) treatments in black band disease found on the 3 species of corals. There were significant differences in the microbial compositions of the separate black band samples taken from the three species of coral (p-value = 0.001). The ordination plot shows clearly defined separation (figure 6).

There were no significant differences in the microbial composition of the coral mucus samples between different treatments (p-value = 0.248). There were significant differences between the mucus microbial compositions among the 3 species (p-value = 0.001).

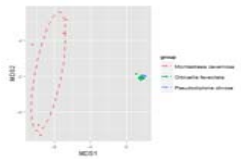

Figure 7. Ordination plot of the bacterial community of coral mucus.

Results

Two genera of bacteria contributed to 74% of the dissimilarity between high and low pH samples grown in marine broth; *Vibrio* (37.89%) and *Propionigenium* (36.05%). Two genus of bacteria contribute to 74% of the dissimilarity between high and low temperature samples grown in marine broth; *Vibrio* (36.57%) and *Propionigenium* (36.43%). In the different pH and temperature regimes the Vibrio genus made up approximately 90% of the black band sample (Table 1,2).

Three genera of bacteria contributed to 50% of the dissimilarity between the low and high pH samples grown on the coral; *Rosiebactrium* (23.26%), *Oscillatoria* (20.94%) and *Clostridiisalibacter* (5.81%). Between the high and low temperature samples from the black band coral samples, the same three genera contribute 50% of the dissimilarity; *Rosiebactrium* (24.22%), *Oscillatoria* (20.31%) and *Clostridiisalibacter* (6.17%).

Four genera of bacteria contributed to 22% of the dissimilarity between the low and high pH mucus samples. *Roseibactrium* (5.73%), *Pseudomonas* (5.69%), *Ruegeria* (5.65%) and *Roseovarius* (5.04%). Four genera of bacteria contributed to 22% of the dissimilarity between the low and high temperature mucus samples. *Pseudomonas* (5.60%), *Roseibactrium* (5.35%), *Roseovarius* (5.28%) and *Ruegeria* (5.27%).

Marine Broth Black Band pH Treatments		
Bacterial Genus	**High**	**Low**
Vibrio	91.07	90.89
Propionigenium	5.85	5.74
Coral Black Band pH Treatments		
Bacterial Genus	**High**	**Low**
Roseibacterium	36.34	30.57
Oscillatoria	23.84	16.17
Clostridiisalibacter	2.77	5.54
Coral Mucus pH Treatments		
Bacterial Genus	**High**	**Low**
Roseibactrium	4.32	8.74
Pseudomonas	4.72	3.41
Ruegeria	6.69	10.72
Roseovarius	7.02	8.66

Table 1. The relative percent composition of the bacterial genus that contribute the most dissimilarity for each type of sample in separate pH regimes.

Marine Broth Black BandTemperature Treatments		
Bacterial Genus	**High**	**Low**
Vibrio	92.079	89.88
Propionigenium	3.85	7.75
Coral Black Band Temperature Treatments		
Bacterial Genus	**High**	**Low**
Roseibacterium	24.75	42.16
Oscillatoria	23.06	16.95
Clostridiisalibacter	6.84	1.48
Coral Mucus Temperature Treatments		
Bacterial Genus	**High**	**Low**
Roseibactrium	5.26	7.59
Pseudomonas	6.56	1.16
Ruegeria	9.18	7.68
Roseovarius	6.20	9.68

Table 2. The relative percent composition of the bacterial genus that contribute the most dissimilarity for each type of sample in separate temperature regimes.

Conclusions

There was a significant difference between mean surface area the pH treatments in the OAsys experiment. This confirms the results of the July 2013 study, and indicates the well water at the Tropical Research Lab had no effect on the results.

The microbial analysis of the July 2013 study showed that there were differences in the microbial community between marine broth grown black band and black band found on the corals, although not significantly different. These bacterial shifts may account for the difference in growth rate in when each are exposed to low ph. *Vibrio* species are know to be coral pathogens, and exploded in the marine broth cultures. There were less *Rosieobacter* and *Oscillatoria* in the low pH of the coral black band, possibly indicating a role in it's slower growth at that pH(7.7).

There were significant difference in the microbial composition black band grown on the three separate species, which indicates that black band has a unique microbial composition based on the coral species it infects.

Acknowledgements and References

Emily Hall for the use of Mote OAsys laboratory, Mote Marine Laboratory staff and interns, Mote Tropical Research Laboratory staff especially Erich Bartells, NSF REU Award: 1156580, MRDNA Laboratories

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