Investigating Coral Bacterial Communities in the Flower Garden Banks

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Abstract

Mesophotic reefs have historically been understudied, leading to a dearth of information on the corals that compose these reefs. Recent advancements in technology have enabled researchers to have greater access to reefs at mesophotic depths, generating a greater interest in the microbial communities in both mesophotic and shallow reefs, in order to determine the relative bacterial community differences. Samples of coral mucus from *Montastraea cavernosa* were collected from Flower Garden Banks National Marine Sanctuary (FGBNMS) in the summer of 2010 and 2011. Bacterial community DNA was extracted from the coral mucus and amplified using LH-PCR. Length Heterogeneity PCR is a technique used to amplify the hypervariable V1 and V2 region of the 16s rRNA gene in order to determine relative base pair length and abundance of microbial communities. During the PCR product verification, contamination became apparent in the gel. Through contamination troubleshooting, the source was determined; however, not soon enough to continue with analyzing the samples. This project will be continued in the future in order to add to the mesophotic bacterial community database. The DNA extraction protocol frequently utilized in the laboratory will be modified to increase DNA yields, PCR conditions will be optimized, and the samples will be fully analyzed.

Introduction

Coral reefs are characterized by high biodiversity and productivity due to the fact that they provide ecosystem services including: nutrient output, protection from waves and currents, and export fish and invertebrate larvae (Moberg and Folke, 1999). Stony corals are known as autogenic ecosystem engineers because they modify themselves and physically build upon the environment in which they reside, providing structural habitat for a myriad of trophic levels (Gutierrez and Jones, 2008). Scleractinian, or reef-building, corals live in a delicate symbiosis with bacteria and algae in the genus *Symbiodinium*, also known as zooxanthellae. The coral host, bacteria, fungi, viruses and zooxanthellae together comprise the coral holobiont. Balance in all aspects of the holobiont is crucial to maintain in order to ensure ecosystem stability (Ainsworth et. al., 2010). Each compartment of the coral is characterized by different, species-specific bacterial community associations (Bourne and Munn, 2005). The bacterial assemblages are vital to the coral's health, playing a critical role in sulfur and nitrogen nutrient cycling and acting as a barrier to disease (Rosenberg et. al., 2007; Lesser et. al., 2004; Raina et. al., 2009). Coral tissues exhibit microhabitat partitioning, creating fluctuating diversity in the bacteria that are associated with the coral depending on the environmental conditions present, such as the amount of light or oxygen available or chemical composition (Ainsworth et. al., 2010).

Declines in shallow coral reef cover over the past three decades have been largely attributed to a suite of anthropogenic stressors. Eutrophication and climate change are known as the principle stressors, and overfishing, dragging anchors, and pollution have led to significant decline in coral health, as well (McDole et. al., 2012). Not only are the physical structures of coral reef communities diminishing, but coral bleaching and disease have negatively impacted many shallow, nearshore coral reef communities throughout the world. While easily-accessible, shallow reefs close to shore have been well studied, evidence suggests the data accumulated from in-shore shallow reefs skews our perception of coral reefs (Menza et. al., 2007). Recent advances in technology have enabled researchers to study habitats, like mesophotic coral reefs, which were previously inaccessible. Mesophotic reefs are defined as deep reef communities (30m to 150m) occurring in low light habitats. Mesophotic reef communities are composed of light-dependent zooxanthellate scleractinian corals, azooxanthellate scleractinian corals, macroalgae, and sponge-dominated communities (Lesser et al., 2009). Due to their depth, mesophotic reefs are believed to have relatively little impact from local anthropogenic stressors, allowing researchers to assess differences between euphotic reefs and mesophotic reefs in order to identify the sources of the stressors that are affecting shallow reef environments (Lesser *et. al.*, 2010). Although these ecosystems are not pristine, they provide an environment in which runoff and recreational carelessness cannot be a contributing factor. Due to the decline in the coral population of shallow reefs, Glynn (1993) proposed that corals of mesophotic depths could potentially serve as a reservior for larvae and species from shallow reefs. In order for this hypothesis to prove accurate, there would need to be little genetic differentiation or a high degree of phenotypic plasticity across depth gradients in each component of the coral holobiont (Madeleine et. al, 2011). The Coral Probiotic Hypothesis proposes that a dynamic relationship exists between symbiotic microorganisms and environmental conditions which brings about selection of the most advantageous coral holobiont (Reschef et. al., 2006). Therefore, if environmental conditions were to change the coral could adapt more quickly to the shifts if

demonstrating the proper selection. Previous studies have shown that some clades of zooxanthellae have a higher tolerance for increased temperatures or light regimes (Rowan, 2004;Frade et. al, 2008) The clades of zooxanthellae that are associated with a greater efficiency for handling temperature and light at mesophotic depths will be most beneficial during environmental change because of their tolerance. Fortunately, *Montastraea* spp. demonstrates a level of constancy within its symbiont distribution regardless of vertical or horizontal location (Frade et al. 2008). Because of the shifting distribution and efficiency of zooxanthellae, some bacterial communities may be more apt to colonize with specific clades of zooxanthellae. On the other hand, the bacterial communities documented with specific clades of zooxanthellae may be a product of similar abiotic conditions versus a preference for a specific clade of zooxanthellae.

This project will determine bacterial community structure within the surface mucus layer (SML). Mucus is secreted at different rates by the coral for a number of reasons, including heterotrophic feeding, sediment cleansing, and environmental stress defense (Brown and Bythell, 2005). Mucus is often referred to as the coral's first line of defense. Varying SML conditions due to the state of the coral are associated with varying bacterial assemblages (Sweet et. al, 2010). While the SML physiologically provides a barrier with its polysaccharide rich layer, the microorganisms that reside within the mucus also provide protection from pathogens by interspecific competition and secretion of antibiotic substances (Rosenberg et. al. 2007). The mucus secreted by the coral polyps varies in thickness, oxygen availability, and chemical composition (Sweet et. al., 2010). The environmental conditions together with the coral's physiological condition determine the microbial community associated with a coral holobiont. On the other hand, changes in the microbial communities may affect the coral physiology (Kooperman et. al., 2007).

Montastraea cavernosa was chosen as the model scleractinian species for this study because of its importance in reef building and its dominance in both shallow and deep coral reefs. *M. cavernosa* is documented to have a wide depth distribution and is one of the most numerous corals at mesophotic depths (Lesser *et. al.*, 2010). *M. cavernosa* samples have been collected through ongoing coral health studies at both the Flower Garden Banks National Marine Sanctuary (FGBNMS). The FGBNMS is a coral reef system that is comprised of two pinnacles on the continental margin on the coast of the Gulf of Mexico. The FGBNMS contains an East Flower Garden Bank (1.02 km^2 of coral reef) and West Flower Garden Bank (0.4 km^2 of coral reef) that originated due to an uplift of underlying salt domes in the Jurassic (Hickerson *et. al*, 2005). The FGBNMS is relatively undisturbed by point-source local anthropogenic effects; the reefs are characterized by relatively low diversity but high coral cover. While the FGBNMS is a deep, unimpacted system, St. Lucie Reef is located at the mouth of the St. Lucie Inlet that is shallow and often impacted by human activities and Indian River Lagoon estuarine discharge. St. Lucie Reef is approximately 7 km long, with coral ranging from 3m-9m deep (Beal *et. al.*, 2012). Both of these sites have been closely monitored for the past 3 years and serve as exemplary reefs for the scope of the project at hand. Comparing the status of the bacterial community associated with the SML from each region, over depth gradients within regions, and among individual sites, can provide insight regarding potential variability in *M. cavernosa* physiology and health status.

Methods

Sampling location and collection. Samples of *Montastraea cavernosa* mucus were collected from stratified random sampling points throughout both the East Flower Garden Bank and the West Flower Garden Bank. At the FGBNMS, the habitat has been categorized into five main types based on depth and dominant species. This study sampled mucus from *M. cavernosa* in the coral reef and coral community habitats. Technical and standard SCUBA divers collected mucus samples from visibly healthy corals larger than 20cm in diameter using a sterile 10mL syringe. The mucus samples were allowed to settle for 20-40 min and the bottom 2mL of each sample was placed into a 2mL Cryovial tube. The samples were transported in a cooler with liquid nitrogen to Harbor Branch Oceanographic Institution and stored at -20°C.

Extraction of DNA from mucus samples. Samples were thawed at room temperature. Bacterial community DNA was extracted from the mucus sample by the bead-beating method using the FastDNA SPIN kit for Soil (MP Bio, Solon, OH) after slight modification of the protocol as modeled after Sekar *et. al.* (2006). Samples were spun in an Eppendorf Centrifuge 5430 for 10 minutes at 14,000 rcf to condense the sample. Approximately 600 µl of the mucus sample was transferred directly into the Multimix Lysing matrix tubes. Samples were bead-beaten using the FastPrep Instrument for 20s at $5.5ms^{-1}$. Then the samples were heated at 70°C in an Eppendorf Thermomixer R heating block for 30 minutes and bead-beaten again for 10s at $5.5ms^{-1}$ to ensure maximum cell lysis. Protein Precipitate Solution was added to rid the sample of proteins, and then centrifuged to create a protein pellet. The supernatant was transferred to a new 2mL tube with 1mL of Binding Matrix to bind the DNA to the silica particles. Once DNA bound to the matrix, the supernatant was removed and the settled silica is transferred to a spin filter tube with500µl of Salt-Ethanol Wash Solution. The spin filter and sample is centrifuged at 14,000 rcf for two minutes. After repeating the cleaning step, DNA was eluted using 100µl DES ultra-pure water. The DNA was quantified using a Nanodrop2000 Spectrophotometer. Extracts were diluted, if necessary, to 2ng/µl and stored at -20°C until PCR analysis.

Length heterogeneity (LH) PCR amplification and gel electrophoresis. The genomic DNA extracted from *M. cavernosa* mucus samples was used for amplification of the V1 plus V2 domains of the 16s rRNA gene. The fluorescently labeled forward primer 27F-6-FAM (5'-6-carboxyfluorescein-AGA GTT TGA TCM TGG CTC AG-3') was used with an unlabeled reverse primer 355R (5'-GGT GCC TCC CGT AGG AGT-3'). PCR reagent mixtures contained: 10xPCR reaction buffer, 25mM Mg mix, dNTPs (0.25 mM each), 20µM of forward and reverse primers, 1.25U of AmpliTaq LD Gold® DNA Polymerase (Applied Biosystems, Grand Island, NY), and BSA (0.1%). The final volume was brought up to 20µl using DES H_20 and the 8ng DNA from stock samples. PCR was conducted in a Peltier thermal cycler with an initial denaturing step of 94°C for 11 minutes, followed by 25 cycles of 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were then quantified using a Nanodrop2000 Spectrophotometer and verified by 1% agarose gel electrophoresis using an ethidium bromide stain and a 2k bp low range exACTGene DNA Ladder. For each gel, the positive control used was a Black Band Disease (BBD) community genome extraction sample collected from *Montastraea faveolata* from the Florida Keys Temperature and Light project in 2009. Gels were imaged with the Protein Simple, Flourchem E

gel imager under UV light. The PCR products were stored in a -20°C freezer to be further sequenced in the future.

Results

DNA was extracted and LH PCR was performed on all of the deep and shallow samples available for Flower Garden Banks from the summer of 2010 and 2011. DNA extraction yields ranged from 0-9.4 ng/µl. The 2010 samples averaged 3.54 ng/µl in concentration; whereas, the West Bank samples from 2011 averaged 2.2 ng/µl and the East Bank samples from 2011 averaged 0.411 ng/µl (See figure 1).

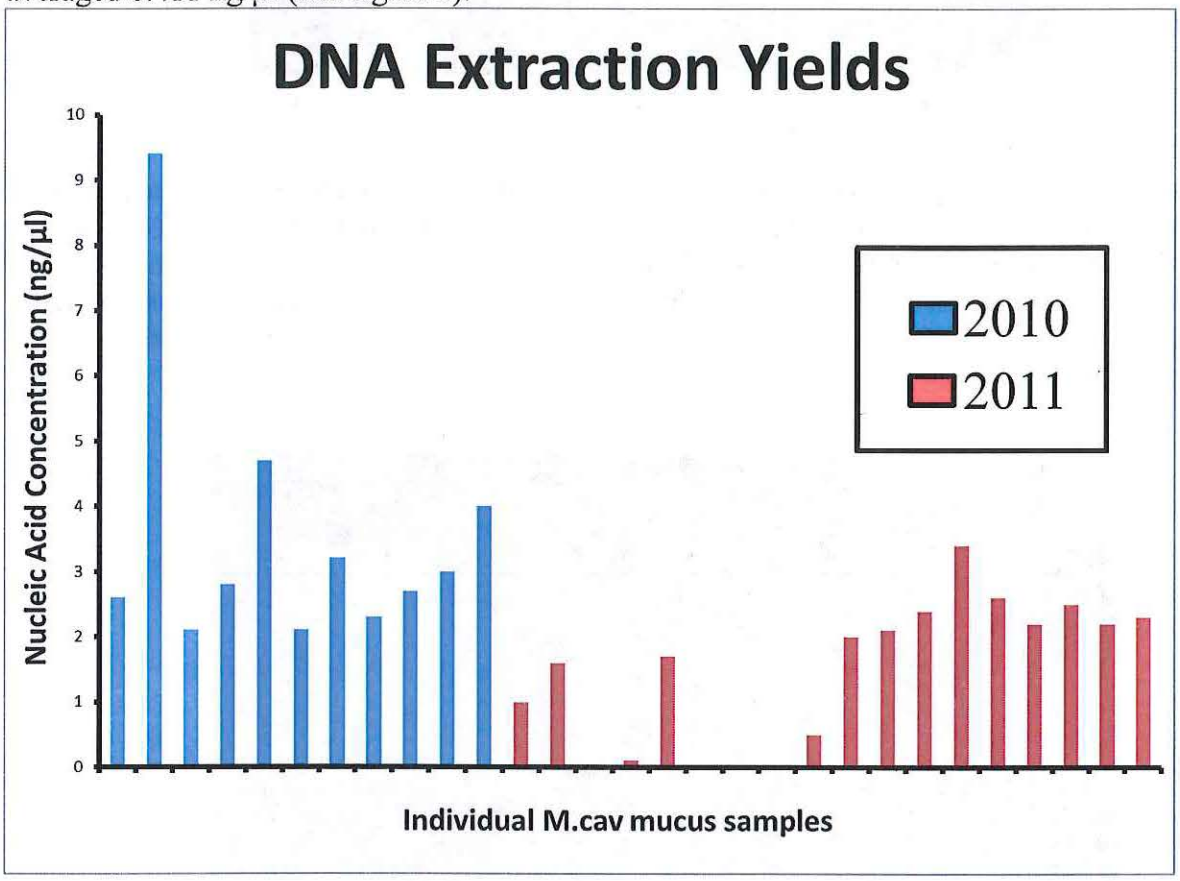

Figure 1. DNA extraction yields from coral mucus samples. The graph above shows the Nucleic Acid concentration in ng/µl from the DNA extractions for the 29 samples from Flower Garden Banks. The blue bars represent samples taken in 2010, while the red bars represent the samples taken in 2011.

The samples from 2011 East Bank were so low in average nucleic acid concentration that a set of duplicate mucus samples were extracted to ensure there was not an extraction error. Extraction of the duplicate East Bank samples averaged 1.011ng/µl. The concentration of DNA from the extractions was lower than the concentration necessary for LH-PCR. PCR was conducted for all samples and the results of the PCR were verified using two techniques: spectrophotometry and

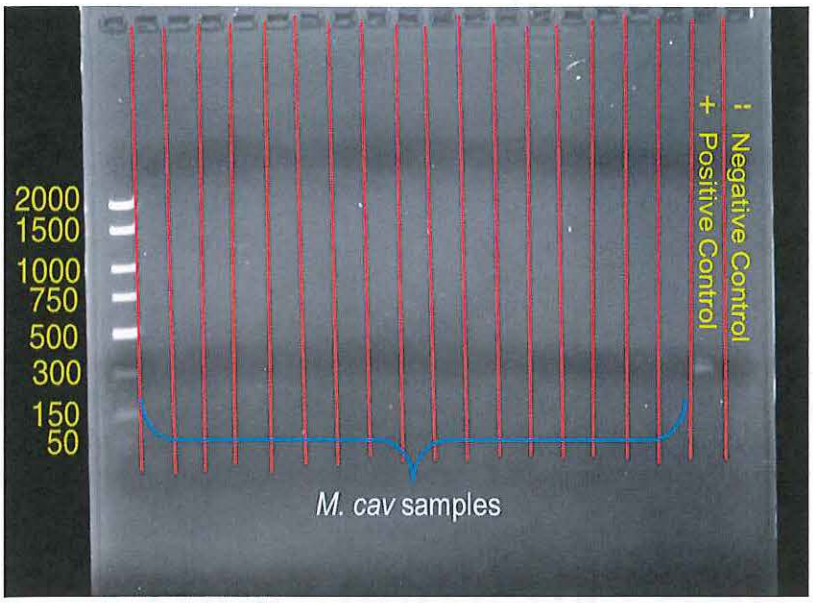

Figure 2. PCR initial trial. The image above shows one gel from initial trials of PCR. No detectable bands were produced.

1% agarose gel electrophoresis. Gel electrophoresis yielded no detectable bands in any of the 29 original samples and the 9 duplicate samples (See Figure 2). During the troubleshooting process for better PCR yields, contamination was found in the negative control of the gel, but not any of the samples. Troubleshooting determined that all of the old reagents working together in the reaction produced faint bands, while replacing any of the old re agents with new ones provided clear, visible bands (See figure 3).

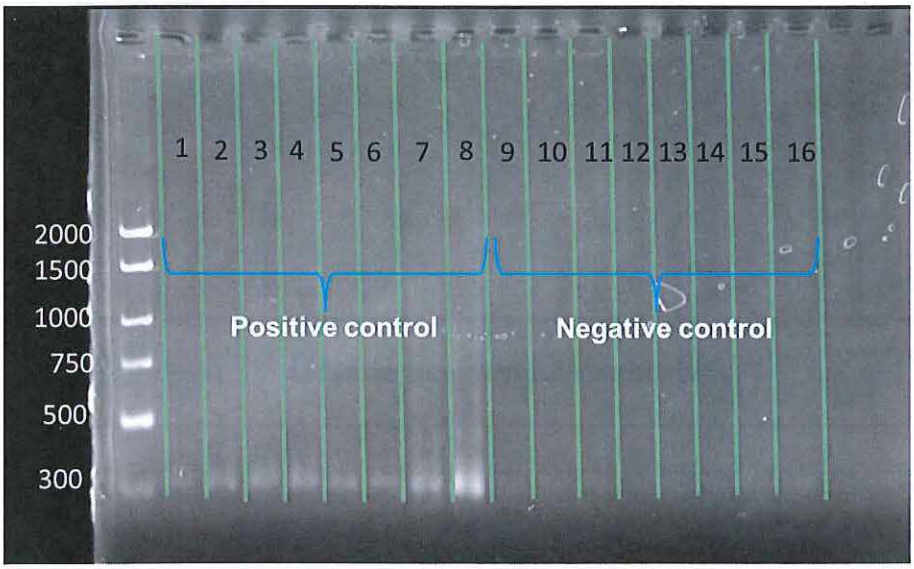

Figure 3. Contamination troubleshoot. Each lane, in the image above, represents a PCR reaction in which one reagent was exchanged for a new one. The positive control block was run with a known sample of Black Band Disease DNA from *Montastraea faveolata* at St. Lucie Reef in 2009. The negative control block is run with plain DES water.

Discussion

Bacterial community profiling could not be completed in the time frame available for analysis due to issues with PCR and DNA extraction. Sample quality was inconsistent because the technical and standard dive teams were unfamiliar with the unique mucus sampling technique and were unable to gather a sufficient amount of mucus sample from each site. An increase in the volume of mucus sampled at each site should provide more consistent sample quality in the future. DNA extraction yields were highly variable, due to sample quality and the DNA extraction protocol utilized. Although the FastDNA Spin kit used in this project is widely accepted for bacterial DNA extraction, in 2010 there was also an attempt to extract bacterial DNA from *Montastraea cavernosa* mucus that experienced similar difficulties (Beal et al. 2012). Protocol modifications and alternate extraction kits will be optimized to produce DNA extractions with greater yields. The MoBio Ultraclean Microbial DNA isolation kit (Carlsbad, CA) will be used to attempt to attain greater DNA yields by following the manufacturer's protocol, as modeled after Morrow et. al. (2012).

While attempting to enhance the PCR product yields from the Flower Garden Banks samples, an increase in cycles, from 25 to 30, and an increase in initial template DNA concentration in the reaction were unable to increase PCR efficiency. These unsuccessful changes may be due to low DNA concentration, but that is not the only contributing factor. Contamination may have also played a role in the lack of the detectable bands. While conducting the contamination troubleshooting, changing both the polymerase (Lane 7 of Figure 3) and the Bovine Serum Albumen (Lane 8 of Figure 3) resulted in bands that were visibly brighter. Overall, determining the source of the contamination increased the PCR product yield.

Montastraea cavernosa is an excellent study species in the sense that its depth distribution is ideal for the scope of the study. On the other hand, *M. cavernosa* proved itself to be a poor study species due to its difficulty with bacterial DNA extraction. As next generation sequencing is being applied more often to molecular biology, potential bias in DNA extraction needs to be addressed. This project will serve as the foundation for my Honors thesis. Future work will focus on improving DNA extraction yields, optimizing PCR conditions, and improving sampling technique.

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