RECOVERY OF XESTOSPONGIA MUTA AND AGELAS CLATHRODES BACTERIAL MICROBIOME SINCE THE 2016 MORTALITY EVENT AT THE FLOWER GARDEN BANKS NATIONAL MARINE SANCTUARY

by

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ABSTRACT

RECOVERY OF XESTOSPONGIA MUTA AND AGELAS CLATHRODES BACTERIAL MICROBIOME SINCE THE 2016 MORTALITY EVENT AT THE FLOWER GARDEN BANKS NATIONAL MARINE SANCTUARY

Charis Ann Peterson

University of Houston-Clear Lake, 2020

Thesis Chair: Lory Z. Santiago-Vázquez, PhD

On July 25, 2016, divers discovered a large localized mortality event at the East Bank of the Flower Garden Banks National Marine Sanctuary. This event killed 80% of invertebrates on the site. This sanctuary is a complex ecosystem where sponges play an essential role as members of the reef. The project analyzes the state of recovery of the bacterial microbiome of the sponges *Xestospongia muta* and *Agelas clathrodes*, since the 2016 mortality event. During the Fall of 2018, divers collected samples of these sponge species from the East Bank, location of 2016 mortality event, and from the West Bank, unaffected during the mortality. A culture dependent approach was used to determine the microbiome of the sponges. The 16S rRNA gene of the cultures was sequenced to identify the bacteria. Alpha-Proteobacteria and Gamma-Proteobacteria were the most common class of bacteria found in *X. muta* and *A. clathrodes* in both the East Bank and

the West Bank. A comparison of the 2018 samples to the communities of the 2016 affected East Bank and unaffected East and West Bank will allow us to determine a potential recovery at the level of bacterial symbionts. Since the microbiome data presented only covers years 2016 and 2018, More research in microbial analysis is needed to determine the overall status of the FGBNMS reef and to establish a baseline for normal conditions.

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INTRODUCTION

Coral Reefs

Coral reefs are amongst the most biodiverse habitats on earth[1]. Coral structure provide habitats for approximately 1/3 of fish[1; 2];[3]. These biodiversity hotspots also protect coastal areas during extreme weather events and provide recreational and cultural benefits to local people[1] and support fisheries [1]. Environmental stressors, including extreme temperatures, depleted oxygen levels, acidification and fluctuations in salinity levels can stress coral reef systems. These stressors appear linked to climate change and can result in coral bleaching, where the host loses their photosynthetic partner. [2]. Human activities , including overfishing and eutrophication, have accelerated the loss of coral systems[4; 5].

Flower Garden Banks National Marine Sanctuary

The Flower Garden Banks National Marine Sanctuary (FGBNMS) is a coral reef located 160km south of the Texas and Louisiana border along the continental shelf in the Gulf of Mexico (figure 1)[6; 7]. The sanctuary has two main banks and a smaller bank. The East Bank (EB) and West Bank (WB) are the larger banks located 20km apart. Stetson Bank is located 50km northwest of the WB[8]. The banks were originally salt domes that have become underwater mounds[9]. NOAA manages this Marine Protected Area[10]. The EB and WB are at depths between 17m and 50m below the ocean's surface. The WB is approximately 5km wide and 11km long. The EB is approximately 5km wide and 8km long[9]. The FGBNMS has over 50% coral cover, making it one of the healthiest coral ecosystems still in the Gulf of Mexico, Caribbean[9], and possibly the world. This is due to its geographical isolation and removal from coastal stressors such as sedimentation and eutrophication. Relatively few divers visit the reef and those that do must have training

for deep diving. The FGBNMS is a critical part of the Gulf Coast economy as a habitat for fish and a location for recreational diving within the tourism industry[9].

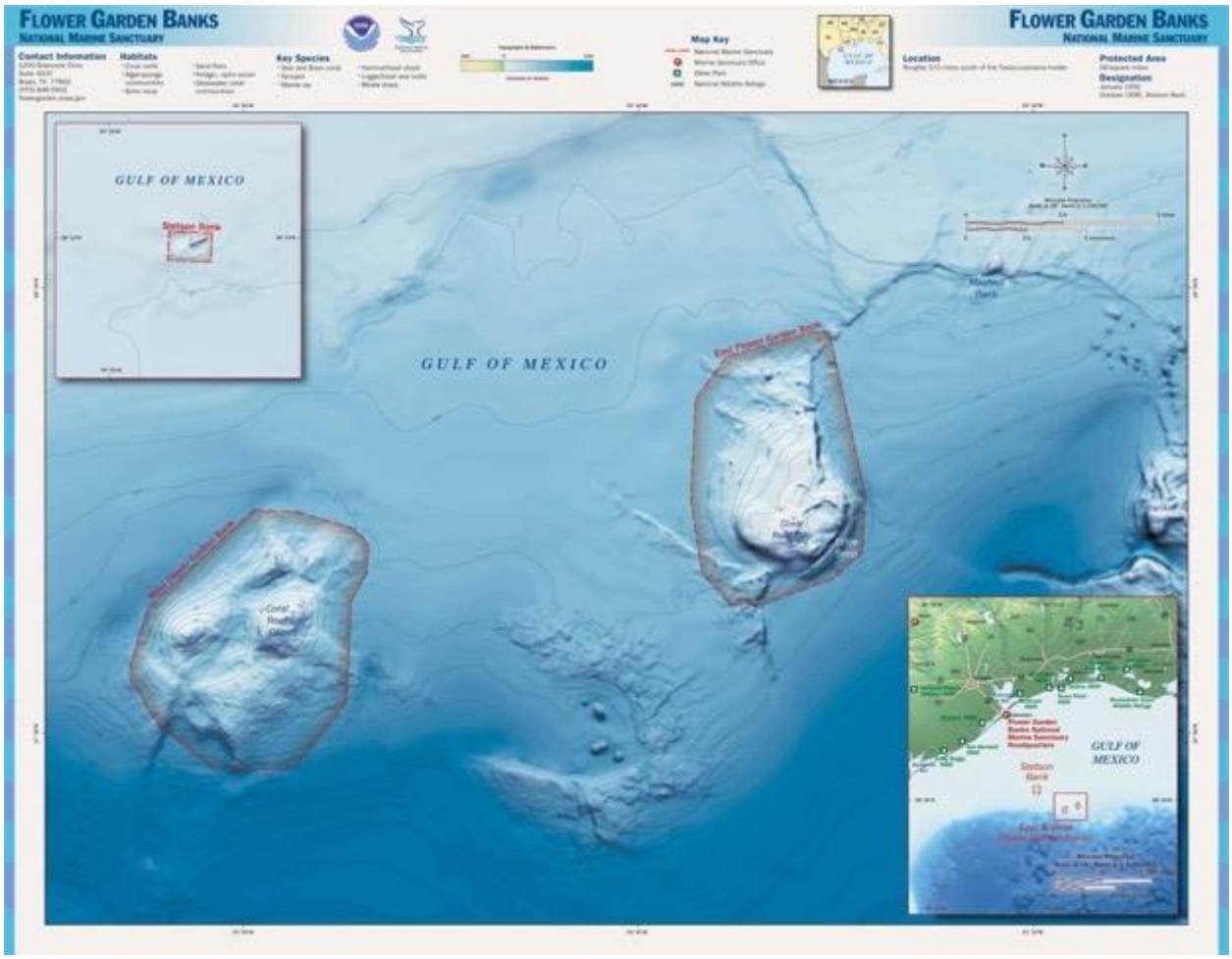

Figure 1. Location of the East and West Bank. This map shows the location, size, and topography of the Flower Garden Banks National Marine Sanctuary. Figure from flowergarden.noaa.gov.

Flower Garden Banks National Marine Sanctuary 2016 Mortality Event

On July 25, 2016, recreational divers at EB reported low visibility and green water. Sponges and coral in some lower portions of the reef were covered in a white mat (Figure 2). Mortality spread over 0.06 km2 of the reef and some areas experienced 80% mortality of invertebrates[6; 7]. All the sessile organisms covered by the mat died (Figure

3). Nearby WB remained healthy[7];[8]. An increase in water flow from Texas rivers as well as an upwelling event created hypoxic conditions, which caused this event[9]. The Mississippi and Atchafalaya rivers had normal water flow, but Texas rivers had an unusually high-water flow at the time. Texas rivers typically account for only 1/5 of the freshwater entering the system but this flow can have a significant impact on the ecosystem. During the event turbid freshwater on the surface of the ocean blocked sunlight and reduced the rate of photosynthesis at depth. At the same time the low salinity water was reaching the EB, there was also an upwelling event at the site. Upwelling is more likely to occur at the EB than the WB because the EB has a steeper seafloor and eddies from the eastern Gulf of Mexico that move westward. This upwelling brought dense, low pH, oxygen-deficient waters to some lower portions of the reef and contributed to the rapid development of hypoxic conditions [9].

Figure 2. White mat. White mat between star and brain coral at the East Flower Garden Banks during July 25, 2016 event. Credit: FGBNMS/G.P. Schmahl

Figure 3. Mortality pattern. Dying star coral colonies show mortality pattern in the East Flower Gardens during July 25, 2016 event. Credit: FGBNMS/G.P. Schmahl

Marine Sponges

Sponges indicate the health of coral reefs[10]. [11]. Due to the sessile nature of sponges, they are well adapted to their environment. Sponges are filter feeders. The water that passes through them is chemically transformed as sponges feed and they excrete by-products and water. Sponges are also highly affected by the physical environment, such as water temperature, dissolved oxygen levels, and pH[12].

Sponges host a large bacterial microbiome. [13]. Between 40% and 60% of sponge biomass is made of symbionts, which are located both intra cellularly and extra cellularly. Symbionts that are on the outer layer of sponges are exosymbionts. Symbionts located in the mesophyll are endosymbionts. Symbionts that permanently reside in the sponge cell or nuclei are called intracellular or intranuclear symbionts[13]. Sponges provide a more nutrient rich and safe habitat for their symbionts. Symbionts help with digestion,

translocation of metabolites and photosynthesis [14; 15]. Sponge microbiome assist their host chemical defenses against predators and are a good candidate for new pharmaceuticals[13]. Sponges produce many secondary metabolites. Bacteria associated with sponges produces some of these bioactive secondary metabolites and some can be might be able to be grown and harvested independently of the sponge host[16].

Sponge Marine Natural Products

Marine organisms produce secondary metabolites to aid in reproduction, to avoid predation, fouling, and competition as well as aid in reproduction[25]. Marine natural products are used in the pharmaceutical industry as antibiotics, anticancer, antiviral, and anti-inflammatory medicines as well as other medical treatments[26]. Sponges are the most important source of these products. Clarhamnoside Rhamnosylated R-Galactosylceramide was found in *A. clathrodes*[26.] Glycosphingolipids, diterpene alkaloids, and bromopyrrole alkaloids was found in *Agelas* species[27]. Xestosaprol F–M (pentacyclic compound), which shows potential for treating Alzheimer's disease[26] ,was found in a *Xestospongia* sp. *and* hundreds of important chemical constituents have been extracted from *Xestospongia* species since the 1970's, including sterols, fatty acids, quinones, terpenoids and alkaloids.[28]

Experimental Subjects

Xestospongia muta (Giant Barrel Sponge) and *Agelas clathrodes* (Orange Elephant Ear Sponge) were analyzed throughout this study because they are easily identified and abundant in the FGBNMS.

Xestospongia muta

X. muta, the giant barrel sponge (class Demospongiae) is long-lived and large, with a height and diameter exceeding 1m[18]. It has a huge barrel-shape with a stone-hard exterior that is often jagged and rough (Figure 4). The species can be found in shades of

gray, brown, and red/pink. The sponge is usually solitary but can have a few smaller individuals growing around its base [19]. *X. muta* is typically found in the Gulf of Mexico, Caribbean, and Florida, along steep slopes at depths between 15m and 40m[19].

Figure 4. X. muta. Large barrel sponge in the Flower Garden Banks National Marine Sanctuary. Credit: flowergarden.noaa.gov

Agelas clathrodes

A. clathrodes, the orange elephant ear sponge (class Demospongiae) is bright orange. This thick rubbery sponge has pits on its surface (Figure 5). It grows in irregular masses. It can encrust large areas of coral reefs or grow in huge ponds. Sometimes it extends from the reef in large flat mats that resemble elephant ears. The sponge inhabits

reef walls in areas with water movement and grows to 2m. *A. clathrodes* can be found throughout the Gulf of Mexico, Caribbean, and Florida, at depths between 10 and 40m[19].

Figure 5. A. clathrodes. Orange elephant ear sponge in the Flower Garden Banks National Marine Sanctuary. Credit: flowergarden.noaa.gov

Bacterial Symbionts of *A. clathrodes* and *X. muta*

The microbiome of *Agelas clathrodes* has not been widely studied when compared to other sponges such as *X. muta*. However, there are reports that include members of the genus. Proteobacteria dominate *Agelas robusta*. The microbiome of these sponges also includes Cyanobacteria, Chloroflexi, Firmicutes, Actinobateria, Acidobacteria, Planctomycetes, Bacteroidetes, and Gemmatimonadetes[17]. Chloroflexi

dominate *Agelas conifera* [18]. These sponges also support populations of Actinobacteria, Proteobacteria and Acidobacteria.

Cyanobacteria, Chloroflexi, and Proteobacteria dominate They also host Acidobacteria, Actinobacteria, and Thaumarchaeota [18-23]. When changes in ocean chemistry stress *X. muta* their microbiome changes. Symbiotic proteobacteria decrease, and cyanobacteria increase[24].

Culture-Dependent Techniques

Culture-dependent techniques allow researchers to identify bacteria down to the species-level and facilitate discovery of pharmaceutically important secondary metabolites. However, culture-dependent techniques identify a lower number of bacteria when compared to culture-independent techniques[25]. Using multiple types of media will allow for a larger number of species to be cultured. In this study, we utilized Marine Agar and 10% Marine Agar. Marine Agar provides nutrients to the bacteria under normal conditions. While 10% Marine Agar will mimic nutrient constraints seen in sponges [26]. These medias were used in 2016[27], shortly after the mortality event occurred, to generate a library of bacterial isolates.

Microbial Identification

Bacteria isolated from *X. muta* and *A. clathrodes* was identified using 16s rDNA and MALDI-TOF analysis. The rRNA gene is widely used for microbial identification and phylogenetic analysis[24]. The because it is present in all bacteria [25]. This gene has highly conserved regions, which are suitable for priming polymerase chain reaction (PCR) reactions, and variable regions, which can be used for identification[28]. Large public databases facilitate the identification of bacterial species by their 16S rRNA gene[29].

Matrix-assisted laser desorption and ionization ion source (MALDI) analyzes large biomolecules[28] and can be used for identification of bacteria. Mass Spectrometry (MS) produces separate gas-phase ions and detects them[30]. [30] Ion Masses (mass-to-charge ratios) are calculated using their Time of Flight (TOF). [30] MALDI-TOF MS was introduced by Michael Karas and Fanx Hillenkamp in the 1980's[31].[31]. Spectra generated from microbial isolated can by compared to a database of references on spectra to provide fast and accurate, strain-level identification[31].

Samples are placed on a target made out of conductive metal[30]. Samples are overlaid with a matrix made of crystalline structures of small organic compounds[30]. This protects the molecules so they can be analyzed and not be fragmented by the laser[31]. A laser beam of UV light introduced energy decomposes the structure of the irradiated crystal which generates a partial cloud where the ions are extracted by an electric field. The ions drift through a vacuum until they reach the detector[30; 31].

The lack of a public database of spectra generated by MALDI-TOF MS results in low identification of some organisms. , particularly bacterial isolated from the environmental samples[32]. Because of the potential cost and time savings, MALDI-TOF MS was used to compare the evolutionary trees built from this technique to DNA sequencing. It can also be used to add the identification from DNA sequencing to the spectra to a database in the future.

Significance

This is only the second project to report a culturable microbiome of *X. muta* and *A. clathrodes* in the Gulf of Mexico. This is the first project to report a culturable microbiome of healthy samples. The microbiomes of 20 individuals of each sponge species was analyzed using this culture dependent technique.

2016 Preliminary Data

Preliminary culturable bacteria data collected in 2016, shortly after the mortality event occurred, showed that sponges in the affected area had an overall decrease in biodiversity (Table 1) compared to what was reported for other *X. muta*[27]. There was also a distinct taxonomical difference between the affected (EB) and unaffected sponges (unaffected EB and WB; Figure 6). The sponge samples were frozen before culturing and the sample size was small. Therefore, this data will be used only for reference and comparisons, not statistical analysis[27].

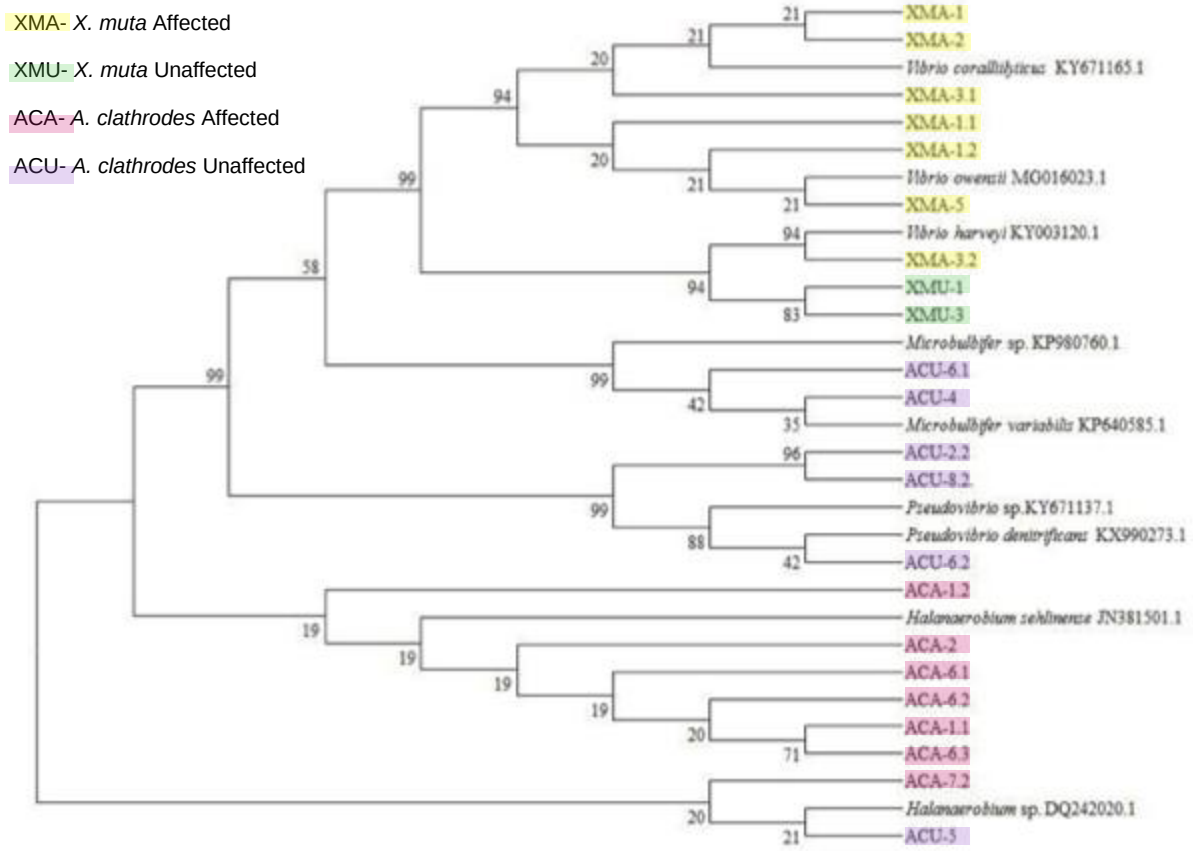

Figure 6. Maximum Parsimony analysis of taxa from culture dependent 2016 samples[27]

Table 1. *Preliminary culture dependent data collected in 2016*[27]

Sponge	Top Phyla in West Bank and unaffected East Bank in 2016	Affected East Bank in 2016
A. clathrodes	Gamma Proteobacteria (*Microbulbifer variabilis*), Alpha Proteobacteria (*Pseudovibrio denitrificans*), Firmicutes (*Halanaerobium sehlinense*)	Firmicutes (*H. sehlinense*)
X. muta	Gamma Proteobacteria (*Vibrio coralliilyticus*)	Gamma Proteobacteria (*V. owensii, V. rumoiens, V. harveyis*)

Hypothesis and Goals: If the EB in the FGBNMS has recovered since the 2016 localized mortality event, then the bacterial microbiome of *X. muta* and *A. clathrodes* will be the same in the East Bank and West Bank in the 2018 samples (Figure 7).

The goal of this study is to determine the state of recovery of the EB sponges since the 2016 event. This will be determined by comparing 2018 microbiomes to those from 2016 using 16s rDNA gene analysis and MALDI-TOF.

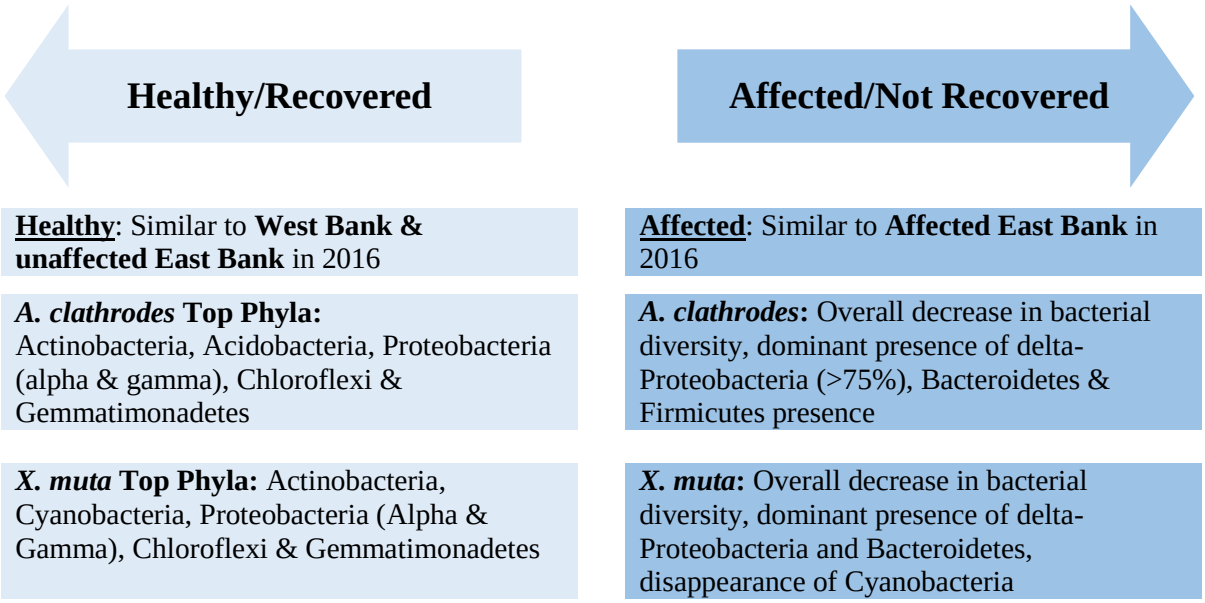

Figure 7. Hypothesis. If the EB Sponges from the FGBNMS have recovered from the 2016 mortality event, the 2018 samples microbiome will be the same in the East Bank and the West Bank samples when compared to healthy 2016.

CHAPTER II:

METHODOLOGY AND MATERIALS

All general lab chemicals and laboratory supplies were obtained from Fischer Scientific Company (www.fishersci.com). The matrix and reagents used for MALDI – TOF – MS were obtained from Sigma Aldrich (www.sigmaaldrich.com). Samples for MALDI – TOF – MS analysis were analyzed at the SEA Facility at Rice University (Houston, TX, USA). Microbial DNA Isolation Kit was obtained from Qiagen (www.qiagen.com). Sample for 16S rDNA sequencing were submitted to Eurofins Genomics (Louisville, KY, USA).

Sponge Sample Collection

Between October 25th and 28th 2018, *A. clathrodes* and *X. muta* samples were collected manually by scuba divers at the Flower Garden Banks National Marine Sanctuary. Dive knives, or scalpels, were sterilized with ethanol and bleach before and after each dive. During the dive, and in between sample collection, divers wiped their knives on different areas of wetsuits and wore gloves. A small section of tissue (~0.5 cm^2) was cut from each individual sponge and placed into its own Ziplock bag filled with seawater. In total, 10 *X. muta* were collected from the EB and 10 from the WB. Additionally, 10 *A. clathrodes* samples were collected from the EB and 10 from the EB. A total of 40 samples were collected (Appendix A).

Bacteria Culturing

When the divers surfaced, the samples were immediately moved to the lab in the collection boat and processed. Samples were rinsed thoroughly with filtered seawater (FSW) and immediately homogenized using a sterile mortar and pestle, and re-suspended in 5ml of FSW[33 34]. A 1:10 dilution was made in FSW and 100µl were plated on Marine Agar and 10% Marine Agar. Marine Agar was made using sterile distilled water and

40.25g/L marine broth. 10% Marine Agar was made using FSW, to maintain salt concentrations close to normal, and 4.025g/L marine broth. To make agar plates 15g/L agar was used. 10µg/ml of cycloheximide was added to both agar types, to prevent fungal contamination. The plates were incubated in the dark at room temperature for 7 days or until significant sized colonies were observed, then cultures were stored at 4°C. Each plate had many morphologically unique colonies of bacteria (Figure 8 and Appendix B). Each type of bacteria was re-streaked until single isolates were observed (Figure 9). The bacteria were stored at 4°C after they were determined to be morphologically pure cultures.

Glycerol stocks were made from each single isolate by inoculating marine broth or 10% marine broth with a single colony. These liquid cultures were incubated in a dark at room temperature for 2 days or until the culture was turbid. 400µl of liquid culture and 600µl of 50% glycerol were mixed and flash-frozen with liquid nitrogen before long term storage at -80°C.

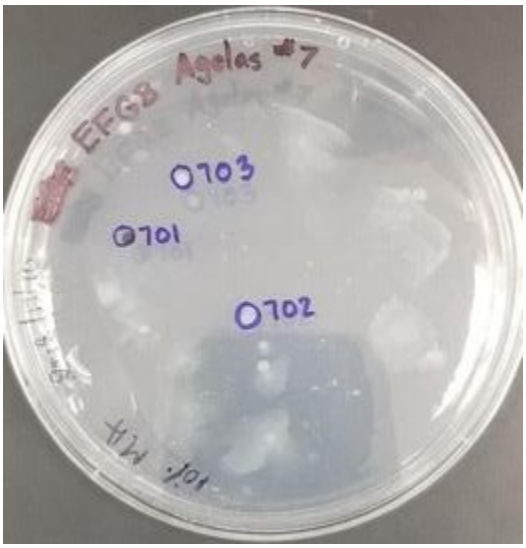

Figure 8. Original 10% Marine Agar plate Sample. Samples ACEB171-18, ACEB132-18, ACEB173-18.

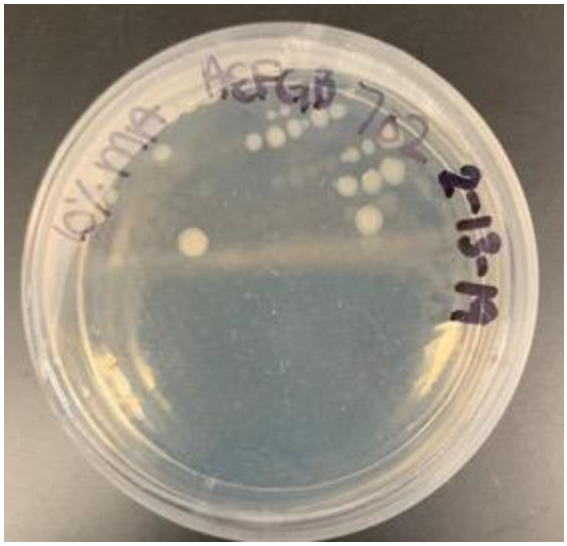

Figure 9. Single Isolate Sample. Sample ACEB172-18 grown on 10% Marine Agar, ready for 16S PCR

16S rDNA Colony PCR

Colony PCR of the 16S rDNA gene was done on all isolated colonies by touching a sterile loop to each colony, making a small "X" on a master grid plate, then swirling the loop in the PCR master mix. The PCR master mix included: 1055F (5'-ATGGCTGTCGTCAGCT-3') and 1392R with a GC cap to improve detection of single-base changes[35] (5'- [CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCC] ACGGGCGGTGTGTAC-3') universal primers with a final concentration of 0.2µM of each primer and 2X GoTaq Green Master Mix (Promega) with a final concentration of 1X. Nuclease free water was used to bring each reaction to a final volume of 25µl.

The thermal cycle profile started with 94°C for 3:00 minutes followed by 20 cycles of the annealing temperature decreasing 0.5°C each cycle starting at 65°C for 45 seconds, 74°C for 2:00 minutes, and 94°C for 1:00 minute (Table 2). This touchdown stage was followed by 10 cycles of 55°C for 45 seconds, 74°C for 2:00 minutes, and 94°C for 1:00 minute. Lastly 1 cycle of 55°C for 45 seconds, 74°C for 5 minutes, and

8°C hold forever. Though out this prosses, the V8 region of the 16s rDNA gene was amplified.

Table 2. *PCR Cycle Profile*

Step	**Temperature**	**Time**
Initial denaturation	94°C	3:00 min
Denaturation	80°C	1:00 min
PCR 1: 20 Cycles		
Annealing	65°C **↓0.5° ea. cycle**	45 sec
Elongation	74°C	2:00 min
Denaturation	94°C	1:00 min
PCR 2: 10 Cycles		
Annealing	55°C	45 sec
Elongation	74°C	2:00 min
Denaturation	94°C	1:00 min
Final Annealing	55°C	45 sec
Final Elongation	74°C	5:00 min
Hold	8°C	forever

DNA Isolation

DNA was extracted and purified from samples that were unable to be amplified with colony PCR. A total of 1ml of liquid, 10% marine or marine broth, was inoculated

with a loop of single isolated colony, incubated at room temperature for 3 days or until the culture was turbid. DNA isolation was done on the pellet of each liquid culture using Microbial DNA Isolation Kit (MoBio UltraClean®)[36].

Agarose Gel Electrophoresis

PCR amplicons (expected size ~350 bp) were analyzed using agarose gel electrophoresis. A 1% agarose gel was prepared with 1X TBE buffer and ethidium bromide with a final concentration of 0.1µg/ml. The gel was loaded with 1µl of Gene Ruler 1KB DNA Ladder from Thermo Scientific and 3µl of PCR product. The gel was run at 130V until the dye was 3/4th of the way down (Figure 10).

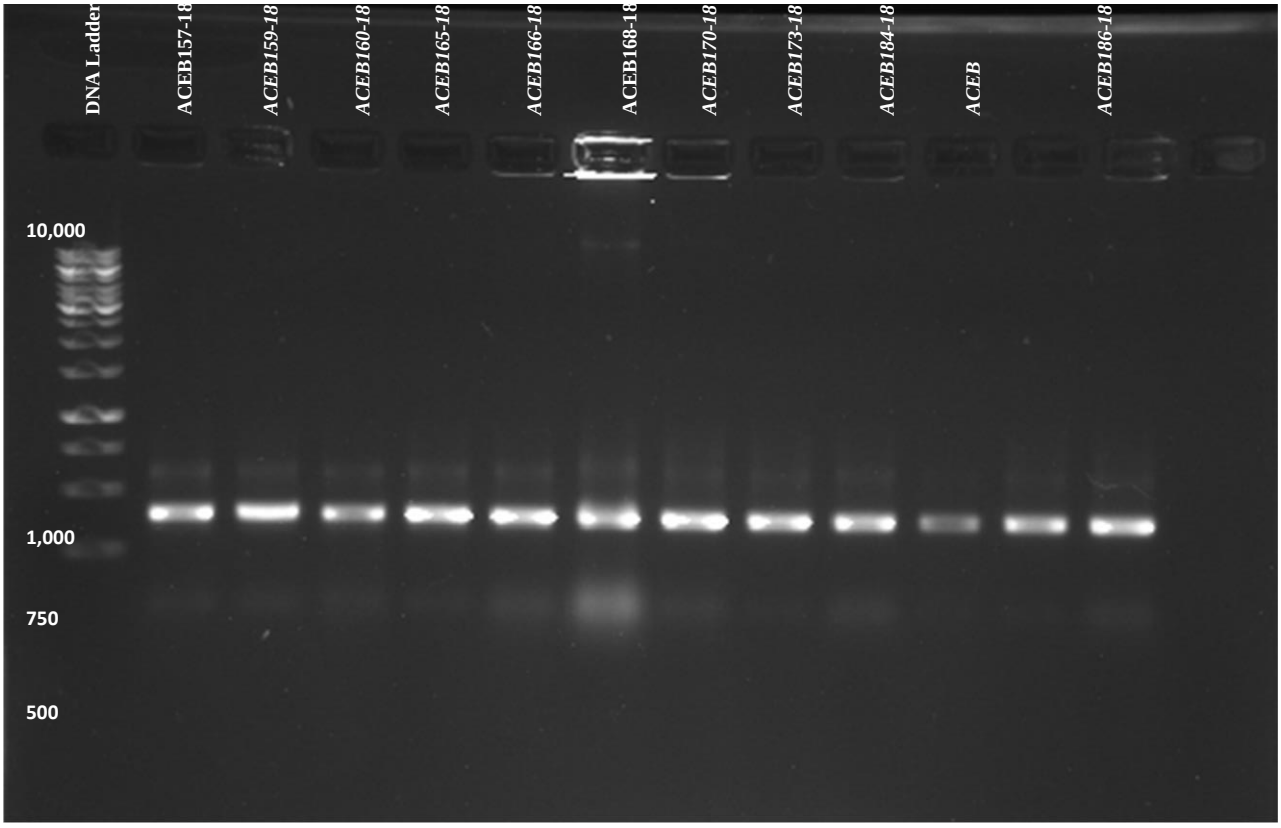

Figure 10. Agarose Gel Electrophoresis Sample. A total of 3µl of colony PCR product was ran on a 1% agarose gel prepared with 1XTBE and 0.1µg/ml final concentration of ethidium bromide at 130V for approximately 1 hour. The first lane contains 1µl of Gene Ruler 1KB DNA Ladder.

Big Dye Sequencing

A total of 10-20µl of each PCR product was loaded onto a green 96-well unpurified PCR plate provided by Eurofins Genomics. 354 Samples were sent to Eurofins Genomics for BigDye Terminator Sanger Sequencing. The samples were sequenced under standard parameters using the 1055F primer [37].

DNA Sequencing Data Analysis

Sequences were manually inspected for quality using Finch TV and. Each sequence was trimmed to begin and/or end with primers. The GC-cap was removed from samples containing the 1392R-GC primer. The closest homologs of the bacteria were determined using GenBank database and the Blastn tool (Appendix C). If the % identity was over 98% samples were identified to the species level when possible. If the % identity was under 98%, samples were identified to their class. Bacteria isolates that were not able to be identified using blast were removed and evaluated for contamination via gram staining. MUSCLE was used to align sequences in MEGAX[38]. The sequences were trimmed to a conserved region at the beginning and end of the sequence. A neighbor-joining tree was built using 10,000 replicates for bootstrapping. Sequences from NCBI were used in the Neighbor-Joining tree as reference. After data analysis, sequences were submitted with GenBank with accession numbers MT474160 – MT474299.

Gram Staining

Gram staining was completed on each colony that had a sequence with mixed or low- quality Sanger sequence signal. A loopful of bacteria was suspended in a drop of sterile water on a microscope slide. The slide was dried at room temperature, then passed through a flame to heat fix. The slide was flooded by Gram's Crystal Violet Solution for 3 minutes, then rinsed with DI water. Next the slide was flooded with Gram's Iodine Solution for 1 minute, and then it was rinsed with DI water. After blotting the slide with a

Kimwipe, the slide was decolorized with 70% ethanol for 20-25 seconds. Then the slide was rinsed with tap water and blotted dry with a Kimwipe. Next, the slide was flooded with Gram's safranin Solution for 1 minute then rinsed in tap water. Slides were viewed under the 100X oil immersion objective. The morphology and Gram status were recorded for each sample, looking for samples with more than 1 morphology or Gram type (Figure 11).

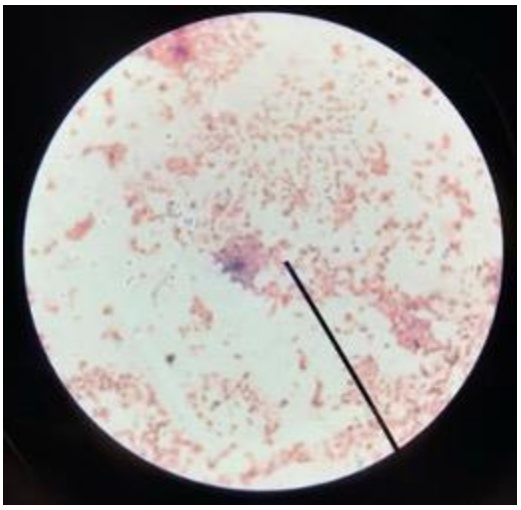

Figure 11. Gram Stain Sample. Sample XMEB097-18 was not a pure culture, Gram-positive and Gram-negative cocci present.

MALDI-TOF MS

A large single colony of freshly grown bacteria was transferred into a tube with 300µl of Ultra-Pure Water (HPLC/MS Grade). This was vortexed thoroughly before adding 900µl of Ethanol (100% HPLC/MS Grade) and the solution was vortexed again. Samples were stored at 4°C for up to 2 weeks. The samples were centrifuged at maximum speed (15,000 rpm) for 2 minutes. The supernatant was decanted, centrifuged (15,000 rpm) for 1 minute, and all of the supernatant was removed. Then, the sample was left at room temperature to complete the evaporation process. A total of 50µl of 70% Formic Acid (HPLC/MS Grade) was added to each sample, then vortexed before sitting

for approximately 5 minutes. A total of 50µl of 100% Acetonitrile (HPLC/MS Grade) was added to each sample before it was vortexed. Samples were centrifuged for 2 minutes (15,000 rpm) and transferred into a clean tube and stored at -20°C. A total of 1µl of the sample was spotted onto the steel target and left to dry. Then overlaid with 1µl of matrix and allowed to dry again[39] (Figure 12). A Bruker Autoflex device with a MALDI source at Rice University was used for mass spectrometry.

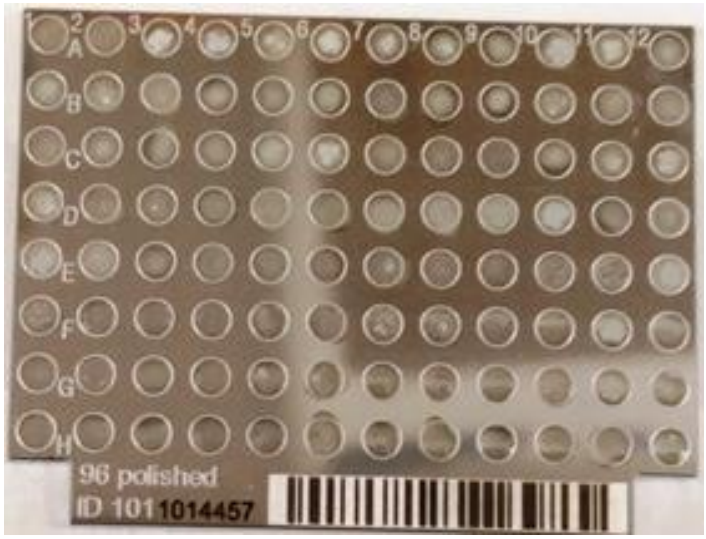

Figure 12. Steel target used for MALDI-TOF MS. This is an example of the target used to spot samples for MALDI-TOF MS and used for mass spectrometry.

MALDI-TOF MS Data Analysis

Analysis of MALDI-TOF MS was done using custom scripts written in R. These scripts utilized functions from the MALDIquant package [40]. The intensity of the spectra was smoothed using the SavitzkyGolay method. The SNIP method was used to remove the spectra baseline. The spectra were aligned using the TIC method. These spectra were then used to construct cluster dendrograms in R, using Ward's method for hierarchical clustering and Euclidean distances. After data analysis, sequences were submitted at massive.ucsd.edu with accession number MSV000085430. (ftp://massive.ucsd.edu/MSV000085430/)

Limitations

The bacteria grown in the lab include only a subset of bacteria associated the sponge. The vast majority of microbes, in any natural system, are not readily culturable (ref). The methods used herein are limited to only bacteria that grow on marine agar and 10% marine agar. Identification using the 16s rDNA gene analyzed with Sanger sequencing is limited to the bacteria that have previously been entered into GenBank (NCBI). The resolution of this gene is limited and different species within a genera can have identical 16S DNA sequences[41].

CHAPTER III:

RESULTS

Initially, 372 bacteria samples were identified morphologically as pure cultures derived from the original sponge homogenates. A total of 354 bacteria samples were sent for DNA sequencing, while the remaining 18 bacteria were unable to be PCR amplified. Out of the samples sent for sequencing, 10 failed and were unable to be sequenced due to low quality DNA. Out of the 344 remaining samples, 83 were removed from the analysis due to poor or short DNA sequences. Out of the sequences submitted, 140 samples were received with high quality sequences that were used to determine the evolutionary relationship of the isolated bacteria. Many of the 140 were duplicates. For the sequences that were present more than once, we selected a representative of the group and used the "Representative Isolate" (RI) for evolutionary analysis. It should be noted that all 140 sequences were analyzed for homology using BLAST (NSBI). The RIs were only used to simplify the phylogenetic tree.

Agelas clathrodes Bacterial Microbiome

We isolated a total of 219 morphologically unique colonies from *A. clathrodes* (121 using MA and 98 using 10% MA; 113 from WB and 106 from EB). Overall, Gamma-Proteobacteria and Alpha-Proteobacteria were the most commonly identified classes in all media and all banks (Figure 13). In addition, we identified one *Dietzia* sp. (Phylum Actinobacteria) in the WB (Table 3).

Xestospongia muta Bacterial Microbiome

We isolated a total of 153 morphologically unique colonies from *X. muta* (68 using MA and 85 using 10% MA; 71 from WB and 82 from EB). Overall, Gamma-Proteobacteria and Alpha-Proteobacteria were the most commonly identified classes (Figure 13) in all media and in both banks (Table 3).

Table 3. *Comparison between 10% Marine Agar and Marine Agar media.*

Species of Sponge	Media	West Bank	East Bank
A. clathrodes	10% MA	Alpha Proteobacteria, Gamma Proteobacteria	Alpha Proteobacteria, Gamma Proteobacteria
	MA	Alpha Proteobacteria, Gamma Proteobacteria Actinobacteria	Alpha Proteobacteria, Gamma Proteobacteria
X. muta	10% MA	Alpha Proteobacteria, Gamma Proteobacteria	Alpha Proteobacteria, Gamma Proteobacteria
	MA	Alpha Proteobacteria, Gamma Proteobacteria	Gamma Proteobacteria

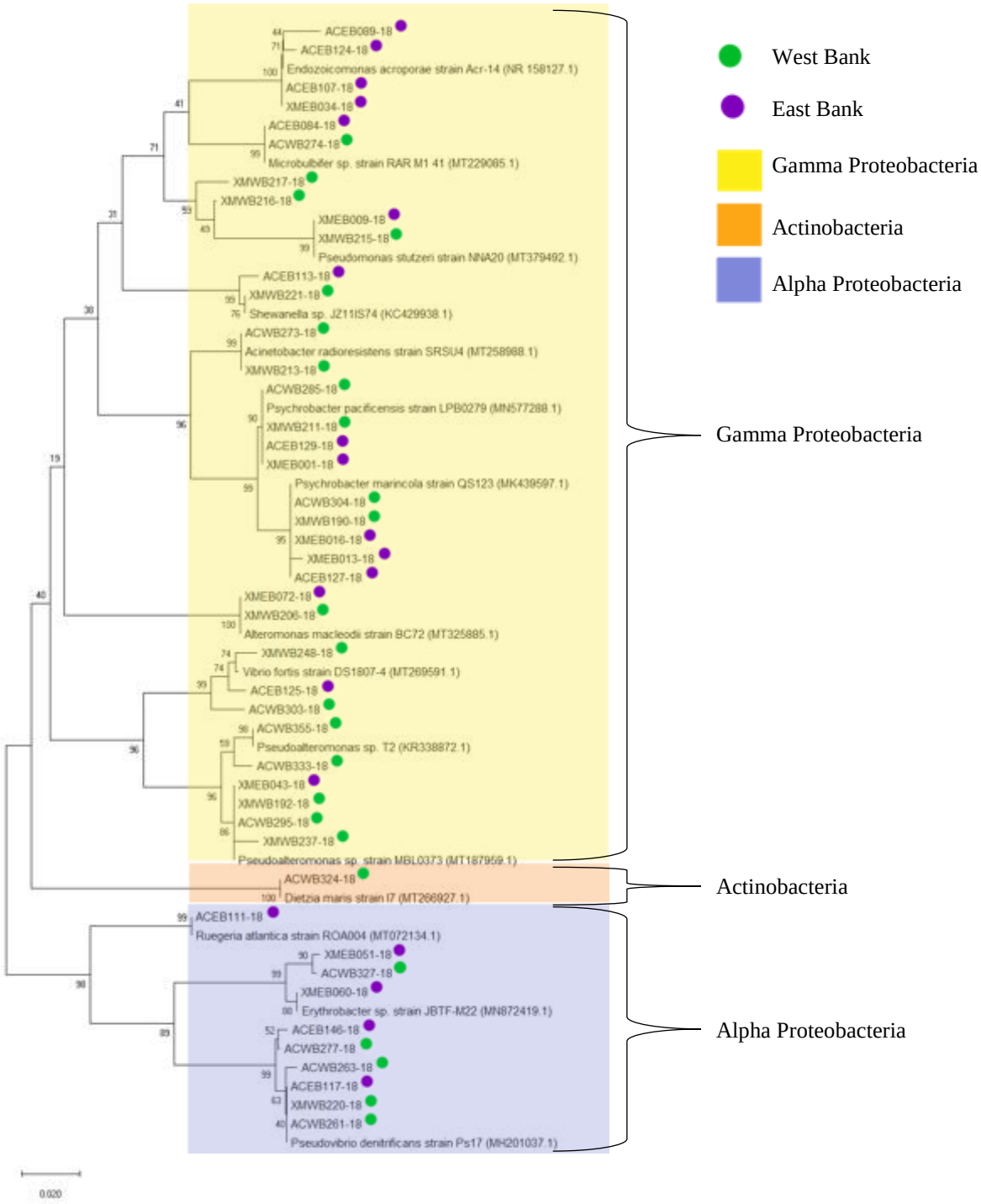

Figure 13. Evolutionary relationship of taxa from DNA sequence. The Neighbor-Joining Method was used to infer the evolutionary history. The bootstrap consensus tree inferred from 10,000 replicates represents the evolutionary history. Branches with less than 50% bootstrap replicates are collapsed. This analysis involved 60 nucleotide sequences. The tree clusters bacteria together by type not sponge species. Analyses were conducted in MEGA.

Gram Staining for Bacterial Colonies that Showed Mixed Signal

A total of 108 bacterial colonies showed mixed signal after Sanger Sequencing. These cultures were Gram-stained and observed microscopically to determine if the culture was pure at least at the morphological level. Keeping in mind, that even if the gram staining showed a pure culture, this could still be a mix of bacteria that morphologically looked similar or identical. A total of 29% of the cultures that were Gram-stained displayed only one morphology. We were unable to determine the purity of 32% of the mixed-signal cultures because the shape or Gram status was not clear. However, 39% of the cultures tested did not have a pure culture; there was more than one shape or both Gram-positive and Gram-negative were present in the same culture (Appendix D).

MALDI-TOF

A total of 96 samples were sent for MALDI-TOF MS analysis. These 96 were selected because the colonies showed healthy growth over time. A total of 75 spectra were used to make a cluster dendrogram due to noise in some spectra. *A. clathrodes* had 15 spectra were from the EB and 14 from the WB. *X. muta* had 31 spectra from the EB and 15 from the WB used in the cluster dendrogram. Cluster analysis of spectra generated by MALDI-TOF MS revealed clusters consistent with species identified by sequencing; however, there were several branches with incoherent topologies (Figure 14).

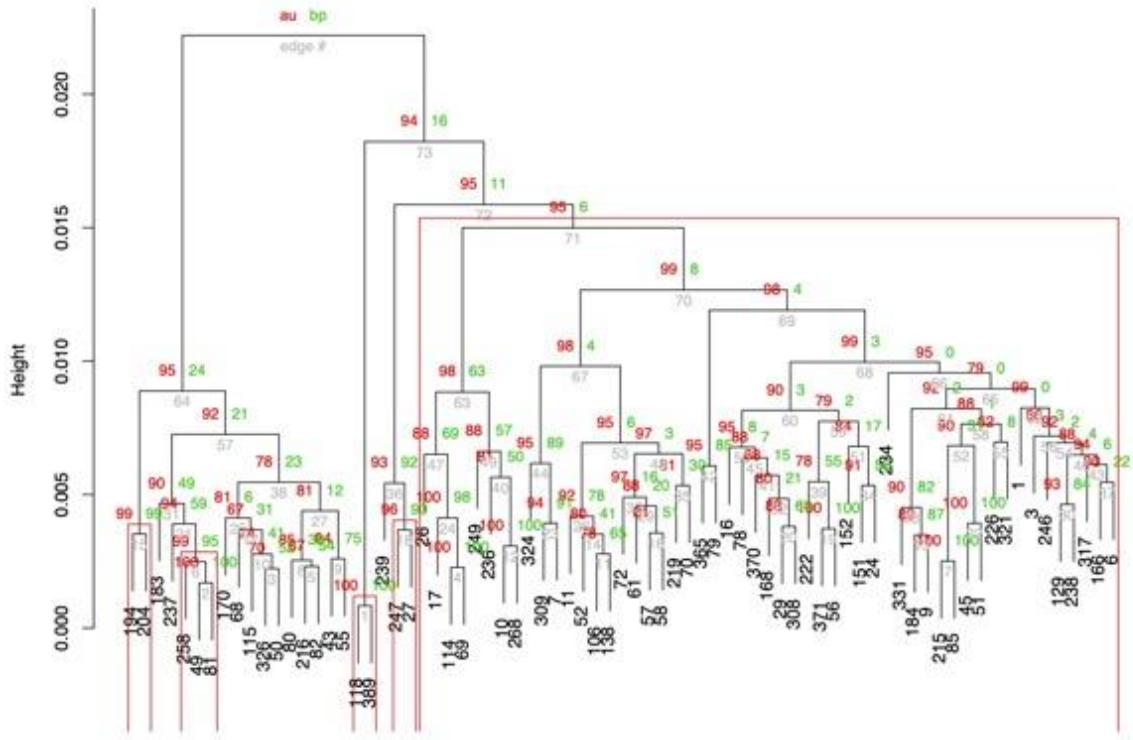

Figure 14. Cluster Dendrogram from MALDI-TOF MS. The p-values are a %. The distance is Euclidean

MALDI-TOF and 16S rDNA Identification Comparison

One large cluster contained mostly *Pseudoalteromonas* sp. and another cluster contained mostly *Psychrobacter* sp. but other gamma proteobacteria were dispersed throughout the dendrogram (Table 4). There were 37 samples identified as Gamma Proteobacteria by 16s rDNA, 7 Alpha Proteobacteria, and 4 Actinobacteria. There was No ID for 27 of the samples.

Table 4. *Comparison of MALDI-TOF MS to 16s rDNA Identification. The samples are in the order of the Cluster Dendrogram (Figure 17) from left to right.*

MALDI-TOF ID	Sample ID	Class	Genus (98% ID on Gen Bank)
194	XMWB194-18	Gamma Proteobacteria	*Pseudoalteromonas*
204	XMWB204-18	Gamma Proteobacteria	
183	ACEB183-18	Gamma Proteobacteria	
237	XMWB237-18	Gamma Proteobacteria	*Pseudoalteromonas*
258	XMWB258-18	No IDID	
49	XMEB049-18	Gamma Proteobacteria	*Pseudoalteromonas*
81	XMEB081-18	No ID	
170	ACEB170-18	Alpha Proteobacteria	
68	XMEB068-18	Gamma Proteobacteria	*Pseudoalteromonas*
115	ACEB115-18	Alpha Proteobacteria	
326	ACWB326-18	No ID No ID	
50	XMEB050-18	Gamma Proteobacteria	*Pseudoalteromonas*
80	XMEB080-18	No ID	
216	XMWB216-18	Gamma Proteobacteria	*Pseudomonas*
82	XMEB082-18	Gamma Proteobacteria	*Pseudoalteromonas*
43	XMEB043-18	Gamma Proteobacteria	*Pseudomonas*
55	XMEB055-18	No ID	
118	ACEB118-18	Gamma Proteobacteria	
389	ACWB289-18	Gamma Proteobacteria	*Microbulbifer*
239	XMWB239-18	No ID	
247	XMWB247-18	Gamma Proteobacteria	*Acinetobacter*
27	XMEB027-18	No ID	
26	XMEB026-18	No ID	
17	XMEB017-18	Gamma Proteobacteria	
114	ACEB114-18	Alpha Proteobacteria	

MALDI-TOF ID	Sample ID	Class	Genus (98% ID on Gen Bank)
69	XMEB069-18	No ID	
249	XMWB249-18	Gamma Proteobacteria	
236	XMWB236-18	Gamma Proteobacteria	*Pseudoalteromonas*
10	XMEB010-18	No ID	
268	ACWB268-18	Alpha Proteobacteria	*Pseudovibrio*
324	ACWB324-18	Actinobacteria	*Dietzia*
309	ACWB309-18	Gamma Proteobacteria	*Psychrobacter*
7	XMEB007-18	No ID No ID	
11	XMEB011-18	No ID No ID	
52	XMEB052-18	No ID No ID	
106	ACEB106-18	No ID No ID	
138	ACEB138-18	Alpha Proteobacteria	*Pseudovibrio*
72	ACEB172-18	No ID	
61	XMEB061-18	No ID	
57	XMEB057-18	No ID	
58	XMEB058-18	Gamma Proteobacteria	*Pseudoalteromonas*
219	XMWB219-18	Gamma Proteobacteria	*Acinetobacter*
70	XMEB070-18	No ID	
365	ACWB365-18	uncultured bacterium	
79	XMEB079-18	No ID	
16	XMEB016-18	Gamma Proteobacteria	*Psychrobacter*
78	XMEB078-18	No ID	
370	ACWB370-18	No ID	
168	ACEB168-18	Gamma Proteobacteria	
29	XMEB029-18	Gamma Proteobacteria	
308	ACWB308-18	Gamma Proteobacteria	*Psychrobacter*

MALDI-TOF ID	Sample ID	Class	Genus (98% ID on Gen Bank)
222	XMWB222-18	Gamma Proteobacteria	
371	ACWB371-18	Gamma Proteobacteria	*Psychrobacter*
56	XMEB056-18	No ID	
152	ACEB152-18	uncultured bacterium	
151	ACEB151-18	Alpha Proteobacteria	
24	XMEB024-18	No ID	
234	XMWB234-18	Gamma Proteobacteria	*Pseudoalteromonas*
331	ACWB331-18	Gamma Proteobacteria	*Psychrobacter*
184	ACEB184-18	Gamma Proteobacteria	
9	XMEB009-18	Gamma Proteobacteria	*Pseudomonas*
215	XMWB215-18	Gamma Proteobacteria	*Pseudomonas*
85	ACEB085-18	Gamma Proteobacteria	
45	XMEB045-18	No ID	
51	ACWB351-18	No ID	
226	XMWB226-18	Gamma Proteobacteria	
321	ACWB321-18	Gamma Proteobacteria	*Pseudoalteromonas*
1	XMEB001-18	Gamma Proteobacteria	*Psychrobacter*
3	XMEB003-18	No ID	
246	XMWB246-18	Gamma Proteobacteria	*Psychrobacter*
129	ACEB129-18	Gamma Proteobacteria	*Psychrobacter*
238	ACWB328-18	Alpha Proteobacteria	
317	ACWB317-18	Gamma Proteobacteria	
166	ACEB166-18	uncultured bacterium	
6	XMEB006-18	No ID	

The top phyla for *A. clathrodes* were Gamma Proteobacteria and Alpha Proteobacteria in both the East Bank and the West bank. The West Bank also had Actinobacteria. The top phyla for *X. muta* were Gamma Proteobacteria and Alpha Proteobacteria in both banks (Table 5).

Table 5. *Top Phyla from 2018.*

Species of Sponge	**Top Phyla in West Bank 2018**	**Top Phyla in East Bank 2018**
A. clathrodes	Gamma Proteobacteria, Alpha Proteobacteria, Actinobacteria	Gamma Proteobacteria, Alpha Proteobacteria
X. muta	Gamma Proteobacteria, Alpha Proteobacteria	Gamma Proteobacteria, Alpha Proteobacteria

CHAPTER IV:

DISCUSSION

The main goal of this project was to determine if the East Bank in the Flower Garden Bank National Marine Sanctuary have recovered since the 2016 EB mortality event by examining the culturable bacterial microbiome of the key reef sponges *X. muta* and *A. clathrodes* collected in October 2018 in both the EB and WB. Bacteria isolated from the EB sponges were compared to the bacteria isolated from the WB sponges in 2018. These results were compared to the culturable microbiome of the same 2 sponges and 2 sites from 2016, and to the culture-independent microbiome that has been reported for these 2 sponges or for member of their genus[42]. The identity of the bacteria was determined using a partial sequence (V8 region) of the 16S rRNA gene. In addition, we used MALDI-TOF to test if this technique could be used as a faster and cheaper alternative to 16S rDNA analysis.

Type of Media

After an analysis of our data, we determined that 10% Marine Agar should be used to culture isolates of *X. muta*, whereas Marine agar is not necessary since we were not able to culture isolates in this media. (Figure 15). Sponge bacterial symbionts accustomed to oligotrophic conditions. It could be hypothesized that for the Proteobacterial isolates, 10% Marine Agar had the right concentration of nutrients for them to thrive. Marine Agar has been used with 10% Marine Agar and several other media types such as ISP medium 2 and R2A medium to increase the type of bacteria that is able to be cultured from sponges representing more of the biodiversity present in the sponge[43]. Culture independent techniques were used to identify a Cyanobacteria community in *X. muta*[36]. BG-11 media was used to culture symbiotic Cyanobacteria in

other organisms[44]. This media should be used to culture and study the cyanobacterial community of *X. muta.*

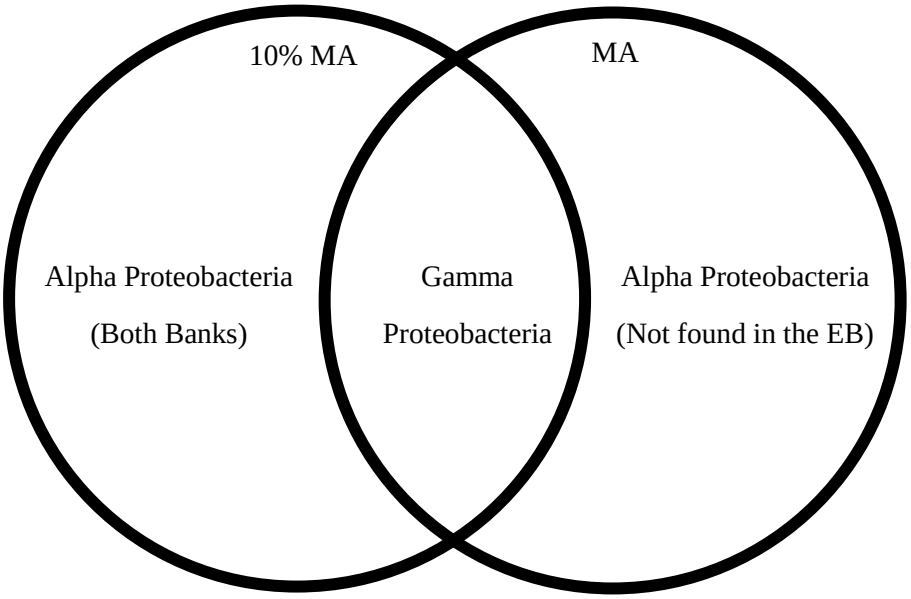

Figure 15. Type of Media X. muta. This is a comparison of the type of bacteria identified that grew in 10% marine agar and marine agar. Samples found in the middle were grown in both medias.

Marine Agar and 10% Marine Agar recovered mostly Alpha Proteobacteria and Gamma proteobacteria. We were also able to isolate one Actinobacteria in Marine agar. The data suggests 10% marine agar should be complemented with other media formulations and not used as the sole media while studying the bacterial biome of *A. clathrodes* (Figure 16). Several Actinomycete isolation medias have been used to culture Actinobacteria from marine sponges. Examples of these media are Actinomycete Isolation Agar, ISP Medium 2 Agar, Starch Casein Agar, and R2A Agar. It should be noted that these media should be adjusted to have 2% (w/v) NaCl when working with marine sponges[45]. Culturing actinobacteria isolated from sponges is important because about 58% of natural products discovered from actinobacteria were originally isolated from sponge bacterial symbionts. These products include the antibacterial Lutoside and the antiparasitic Butenolide[46].

It should be noted that there were a number of isolates that were either not sequenced or that the sequencing reaction produced poor quality sequences. The identity of these cultures is unknown and could have included a more diverse microbiome. There are several reasons for these missing sequences. The number of bacterial samples submitted for DNA sequencing decreased from the number of bacteria samples isolated from the sponge homogenate because we could not PCR-amplify the DNA of 18 samples. This could be due to the 4-month storage of the bacteria plates grown from the sponge homogenate before re-streaking for single isolates. Samples that had poor sequence signals were removed from further analysis. The samples with poor DNA sequences or unreliable identification were Gram-stained. The samples that had pure cultures, according to the Gram stain data, were unable to produce good DNA sequencing results and identification for unknown reasons. Samples that did not have a pure culture were unable to produce a good DNA sequence because they had more than one type of bacteria present. If Sanger sequencing was done on more than one species in a reaction results in overlapping DNA base pairs and the species were unable to be identified. The purity of some samples was unable to be determined because the shape or Gram status was not able to be determined. Gram staining was done after DNA sequencing on samples with poor DNA sequences to determine if the sample was a mixed culture. Gram stains should be completed before DNA sequencing and MALDI-TOF MS in the future. Additionally, samples that have a mixed culture should not be analyzed.

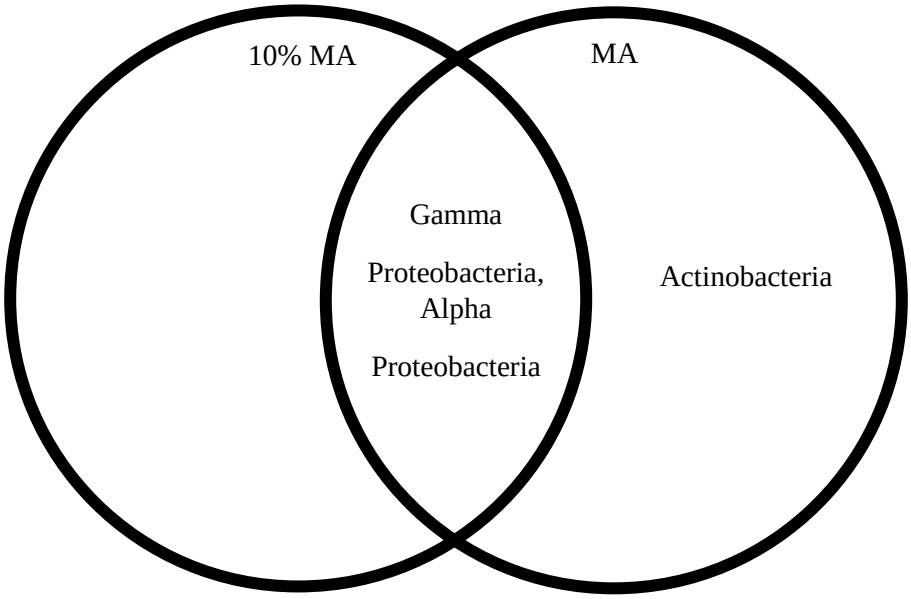

Figure 16. Type of Media A. clathrodes. This is a comparison of the type of bacteria identified that grew in 10% marine agar and marine agar. Samples found in the middle were grown in both medias.

Our results agree previous reports that marine agar selects for Alpha Proteobacteria[36]. In our data, while there were a high number of Alpha Proteobacteria, the majority of our cultures were Gamma Proteobacteria, therefore this media also selects for this class. Several more types of media should be incorporated into this study to culture a wider variety of bacteria. Actino agar, fluid thioglycolate agar, delicious agar, and charcoal agar isolate a more diverse bacteria community when compared to Marine agar. Delicious + ps and basic agar grow even more types of bacteria than those listed above[47]. The use of more types of media will allow culture dependent techniques to identify a larger variety of bacteria. Finally, the storage of original plates, made from sponge homogenates in October 2018, for several months before preparing isolates could create a bias favoring the culture of Proteobacteria and minimizing the culture of more sensitive strains.

Comparison of Bacteria Presence in 2018 with 2016 and the microbiome of *X. muta* and *A. clathrodes*

Alpha Proteobacteria and Gamma Proteobacteria dominated the libraries of isolates from samples of *X. muta* collected in both the East and West Bank (Figure 17). These classes are commonly associated with *X. muta* therefore these results were not unexpected [22]. However, there are other classes known to associate with *X. muta* that were not present in our cultures. These include Chloroflexi, Cyanobacteria, Poribacteria, and to a lesser extent, Acidobacteria, Actinobacteria, and Thaumarchaeota[18; 20-23; 33; 48]. The cyanobacteria' symbionts are unique because they are photosynthetic and live inside the cells of the sponge in a protected environment[49]. In the study, the bacteria were grown outside of the sponge in agar medium as well as in the dark. The Cyanobacteria did not have any light for photosynthesis or its host for protection and nutrients. It is not surprising that Cyanobacteria were not grown the given research conditions. Another example are the Chloroflexi. While these are highly abundant in sponges (20-30% of total microbiome), only a few members have been cultured therefore their culture conditions are not well understood[50]. Culture-independent techniques can identify these bacteria; however, their cultures conditions remain a challenge for most except for the Proteobacteria.

Immediately after the die off in 2016, cultures of *X. muta* had only Gamma Proteobacteria and Firmicutes, but no Alpha Proteobacteria. We only detected Alpha Proteobacteria in 2018 samples. This could be due to a larger number of sponge samples collected or improvements in culturing methods. It is also possible that the Alpha Proteobacteria were not present in large numbers in the stressed microbiomes of *X. muta* but their populations were restored in 2018. After all, sponge Proteobacteria are known have an enrichment of CRISPR and other defense-related mechanisms in marine

sponges[51]. In 2018, Alpha Proteobacteria and Gamma Proteobacteria was cultured from both banks in *A. clathrodes*. Actinobacteria was found in the WB in 2018 but not in 2016[27]. Actinobacteria are known producers of secondary metabolites and these may have had a protective presence in 2018 but may have not been present in affected samples in 2016.

In 2016 there was Firmicutes in *A. clathrodes*. Affected sponges in the EB had *Halanaerobium sehlinense* which is strictly anaerobic[52]. The *A. clathrodes* this bacterium was cultured from experienced an anoxic event. *A. clathrodes* in the EB are no longer experiencing this stress and this type of bacteria is no longer present due to the change in environmental conditions.

Vibrio coralliilyticus cultured from *X. muta* in 2016 is a coral pathogen[53]. *V. owensii* cultured from affected *X. muta* from the EB in 2016 causes disease in crustaceans and coral[54; 55]. *V. harveyis* was found in from the EB in 2016 and in WB *A. clathrodes* in 2018. *V. harveyis* causes disease in many marine species including fish, sharks, shrimp and sea cucumbers[56]. *V. fortis* cultured form *X. muta* from the WB in 2018 has been found in diseased sea urchins[57]. The presence of *Vibrio* species in the FGBNMS sponges could indicate the ecosystem has not recovered fully from the 2016 mortality event or that the presence of *Vibrio* species is not always detrimental to sponges.

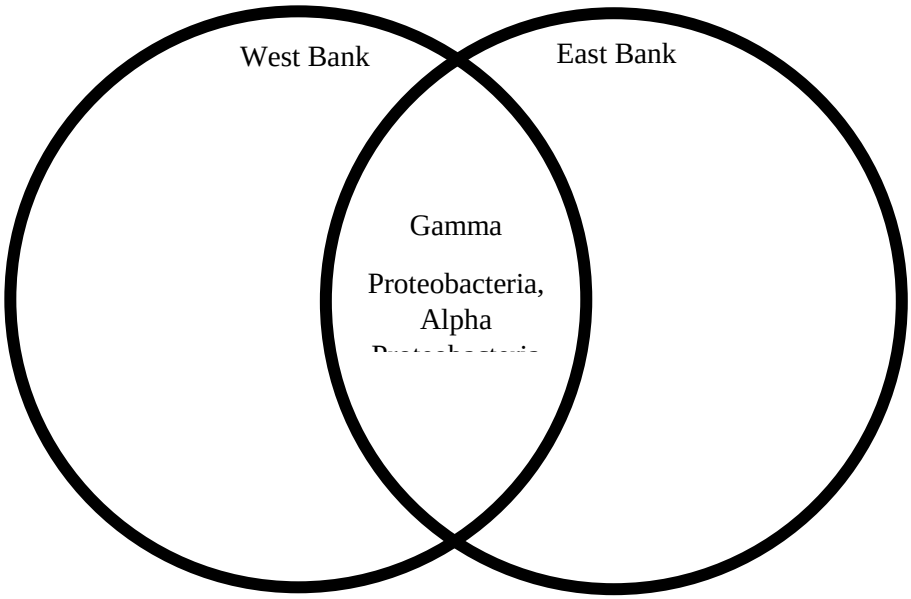

Figure 17. Bacteria present X. muta. This is a comparison of the type of bacteria identified that was found in X. muta in the East Bank and the West Bank. Samples found in the middle were found in both banks.

Alpha Proteobacteria and Gamma Proteobacteria were the most common bacteria found in *A. clathrodes* in the East and West Bank (Figure 18). *Agelas sventres,* collected near Substation Curaçao, contained Alpha Proteobacteria and Gammaproteobacteria[58]. The presence of these classes in other healthy sponges suggests that Alpha Proteobacteria and Gamma Proteobacteria are a natural part of many sponge bacterial biomes. Research on the bacterial biome of *A. clathrodes* is very limited. Other members of the genus have been investigated for their bacterial symbionts. In addition to member of Proteobacteria Phylum, classes Firmicutes, Actinobacteria, Chloroflexi, Thaumarchaeota also present in other *Agelas spp.* from the Florida Keys, Belize, and the Central Amazon Shelf[18; 59; 60]. As with *X. muta*, culture conditions need to be expanded to be able to support the growth of these other bacteria.

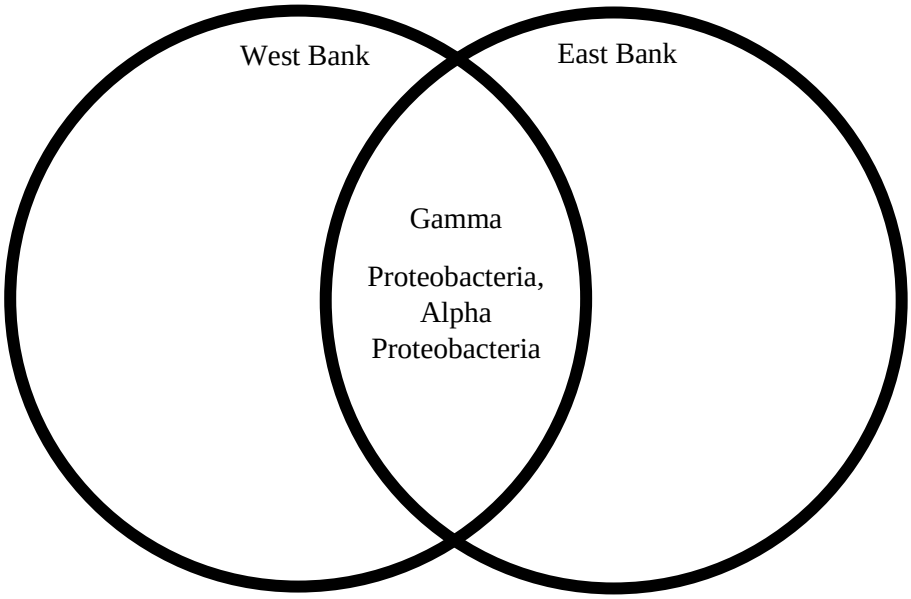

Figure 18. Bacteria A. clathrodes. This is a comparison of the type of bacteria identified that was found in A. clathrodes in the East Bank and the West Bank. Samples found in the middle were found in both banks.

Members of the Gamma Proteobacteria, prominent in both sponges, have many different functions. *Pseudoalteromonas* are adapted to cold conditions and produce secondary metabolites with strong antimicrobial and antitumor activity[61]. While some *Psychrobacter* are infectious to humans[62]. Some *Erythrobacter*, a type of Alpha Proteobacteria, found in marine ecosystems are photo heterotrophs[63]. Some *Pseudovibrio* are adapted to live in close association to sponge hosts. They can produce a wide variety of metabolic compounds, participate in heavy metal detoxification, and have antimicrobial activity[63; 64]. The majority of bacteria identified has been found in marine marine systems. Prior to this study, a few types of bacteria were found in feces, soil, and other locations (Appendix C).

MALDI-TOF

A total of 96 bacterial samples were randomly chosen for MALDI-TOF MS.. The spectra collected from this study will be used to generate a a database for identification.

This databased will be referenced using near intact 16S rRNA gene sequences. This will provide adequate resolution of the isolates and allow for the species to be identified in future studies of sponge bacterial biomes as well as other marine bacteria. Because a database was not used in this study the type of bacteria could not be determined using MALDI-TOF. The MALDI-TOF dendrogram did not cluster the bacteria together by type of bacteria identified using DNA sequencing; however, these methods generally concur[65] and MALDI-TOF has been used to identify Alpha Proteobacteria and Gama Proterobacteria in the past[66; 67]. To address this, near intact sequences 16S rRNA will be used to define reference spectra.

The cost of the MALDI-TOF MS instruments and software is similar to the cost of DNA-sequencing systems. The cost of reagents per sample has been estimated at $0.50. PCR, the first step in BigDye sequencing costs approximately $0.70 for 90bp[68]. MALDI-TOF MS is also fast, taking only approximately 5 minutes per isolate for identification[69]. MALDI-TOF MS should be done before DNA sequencing because some bacteria might be able to be identified in the existing databases. It can also be used for dereplication of samples before DNA sequencing[70].

Health of the Flower Garden Banks National Marine Sanctuary

The bacteria found in *X. muta* did not differ much between the East Bank and the West Bank. The biodiversity decreased from 2016 to 2018. This suggests the affected East Bank is recovering. *A. clathrodes* had a few bacterial colonies that differed from the East Bank and the West Bank. All of the classes that were only present in one location only had one individual isolate identified. Further research is needed to determine if the classes are beneficial or harmful to *A. clathrodes*. In general, *A. clathrodes* had similar bacterial biomes in the East Bank and the West Bank when looking at the most dominant classes. The bacterial biomes from the East Bank and the West Bank have shifted since

the 2016 die-off. This implies that the affected East Bank is recovering. The data collected in this study can be used as a baseline of seemingly healthy and unaffected *A. clathrodes and X. muta* in the FGBNMS. More studies and monitoring are needed assess the health of the FGBNMS and to determine the culturable bacteria biome reference of these two sponges. Marine sponges are just one aspect of the complex ecosystem that makes up the FGBNMS. More research is needed to determine the overall status of recovery of the East Bank.

In September 2016 corals in the East Bank and the West Bank experienced a severe bleaching event due to higher than normal sea temperature. The East Bank had higher temperatures for longer and more extensive bleaching than the West Bank. This bleaching event did not significantly decrease the mean coral cover in either location[71]. In late August 2017, the eye of Hurricane Harvey hit land fall on the Texas cost near the FGBNMS. This extreme weather event reduced the surface salinity levels and caused sub-lethal stress to coral[72].

Future Research Direction

This study is a part of a larger project looking at the cause and effects of the 2016 die-off in the Flower Garden Banks National Marine Sanctuary. Research teams across the United States are looking at different organisms and parameters including coral, sea urchins, and ocean chemistry. Our lab is also using next-generation sequencing to determine the type of unculturable bacteria in the collected sponge samples.

The goal of this research is to continue to monitor the health of the Flower Garden Banks National Marine Sanctuary. This will be done by collecting *A. clathrodes* and *X. muta* samples each fall as described in the methods above. The MALDI-TOF MS data collected from this study will be placed in a database using the 16s rDNA for identification. This will allow for quick and inexpensive identification of the common

bacteria found in *A. clathrodes* and *X. muta*. Samples from future collection will be able to be added to this database as well.

Continuing to annually monitor the bacterial biome of *A. clathrodes* and *X. muta* at the FGBNMS, will allow a baseline of normal to be created. One collection period of samples is not enough to determine this baseline for certain. Establishing the baseline of the bacterial biome will help monitor the health of the reef on an annual basis. If an unusual event happens in the future, similar to the mortality event in 2016, this baseline could be used to determine how bad the reef will be affected and possible causes of the event.

The bacteria cultured in this study should be investigated for their natural products and secondary metabolites. The most important marine source of biologically active natural products come from sponges. Many of these products appear to be produced by bacteria[73]. It is more sustainable to investigate sponge-derived natural products from cultured bacteria than from marine sponges[73]. Antimicrobial, immunomodulatory, and anti-parasitic activity has been found in in bacteria associated with marine sponges [74; 75]. Sponges, and their symbionts, have also been shown to have antioxidant, anticoagulant, antihypertensive, anti-inflammatory, and anti-cancer properties[76]. The bacterial isolates from this study should be investigated for these properties.

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APPENDIX A:

SPONGE SAMPLE COLLECTION OCTOBER 2018

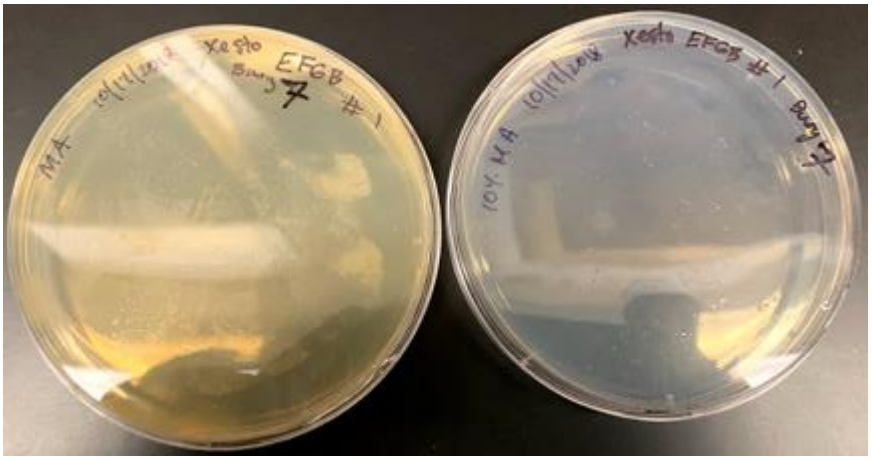
Sponge samples were collected between October 25th and 28th 2018. Species: A; *Agelas clathrodes*, X; *Xesto muta.* Health State: L; Lesioned, A; Affected, H; Healthy. Site: E; East Bank, W; West Bank. Date: 8; Oct2018. Genotype: L; Lory's method.

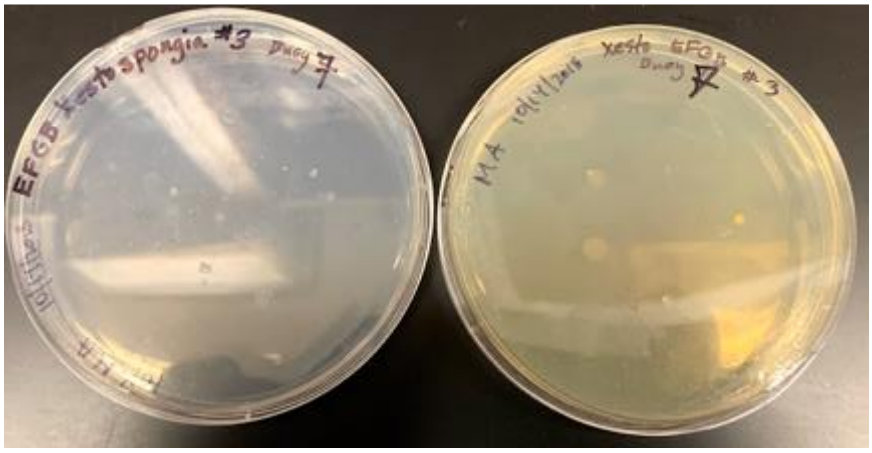
Original ID	QIIME ID	Health State	Site	Buoy
Santiago.XMEFGB.Minus.9	XHE81L	Healthy	East	7
Santiago.XMEFGB.Minus.2	XHE812L	Healthy	East	7
Santiago.XMEFGB.Minus.1	XHE83L	Healthy	East	7
Santiago.XMEFGB.Minus.10	XHE84L	Healthy	East	1
Santiago.XMEFGB.Minus.3	XHE85L	Healthy	East	1
Santiago.XMEFGB.Minus.4	XHE86L	Healthy	East	1
Santiago.XMEFGB.Minus.5	XHE87L	Healthy	East	1
Santiago.XMEFGB.Minus.6	XHE88L	Healthy	East	1
Santiago.XMEFGB.Minus.7	XHE89L	Healthy	East	1
Santiago.XMEFGB.Minus.8	XHE810L	Healthy	East	1
Santiago.XMWFGB.Minus.08	XHW81L	Healthy	West	2
Santiago.XMWFGB.Minus.09	XHW82L	Healthy	West	2
Santiago.XMWFGB.Minus.10	XHW83L	Healthy	West	2
Santiago.XMWFGB.Minus.01	XHW84L	Healthy	West	2
Santiago.XMWFGB.Minus.02	XHW85L	Healthy	West	2
Santiago.XMWFGB.Minus.03	XHW86L	Healthy	West	2
Santiago.XMWFGB.Minus.04	XHW87L	Healthy	West	2
Santiago.XMWFGB.Minus.05	XHW88L	Healthy	West	2
Santiago.XMWFGB.Minus.06	XHW89L	Healthy	West	2
Santiago.XMWFGB.Minus.07	XHW810L	Healthy	West	2

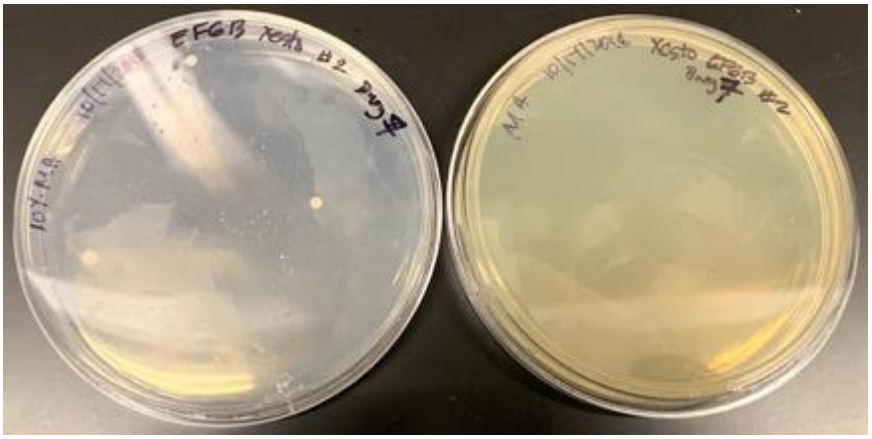
Original ID	QIIME ID	Health State	Site	Buoy
Santiago.A.Minus.EFGB.Minus.02	AHE81L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.03	AHE82L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.04	AHE83L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.05	AHE84L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.06	AHE85L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.07	AHE86L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.08	AHE87L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.09	AHE88L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.10	AHE89L	Healthy	East	7
Santiago.A.Minus.EFGB.Minus.01	AHE810L	Healthy	East	7
Santiago.A.Minus.FWGB.Minus.03	AHW81L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.04	AHW82L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.05	AHW83L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.06	AHW84L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.07	AHW85L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.08	AHW86L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.09	AHW87L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.10	AHW88L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.01	AHW89L	Healthy	West	2
Santiago.A.Minus.FWGB.Minus.02	AHW810L	Healthy	West	2

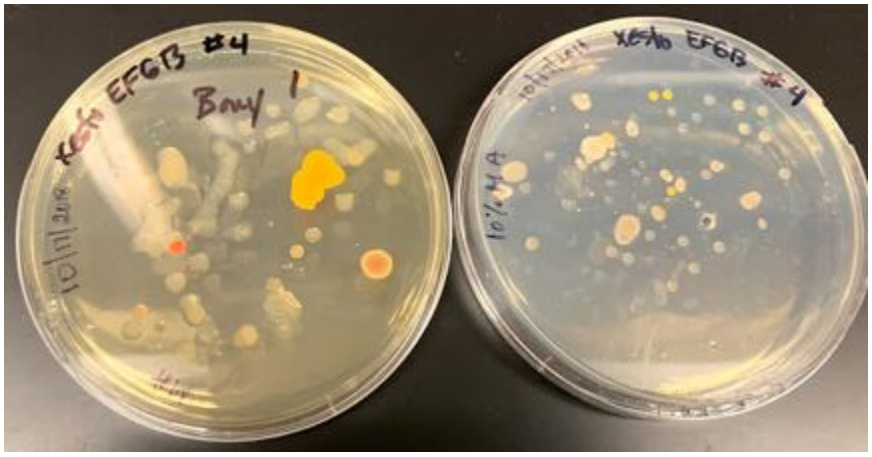
APPENDIX B:

AGAR PLATES MADE FROM SPONGE





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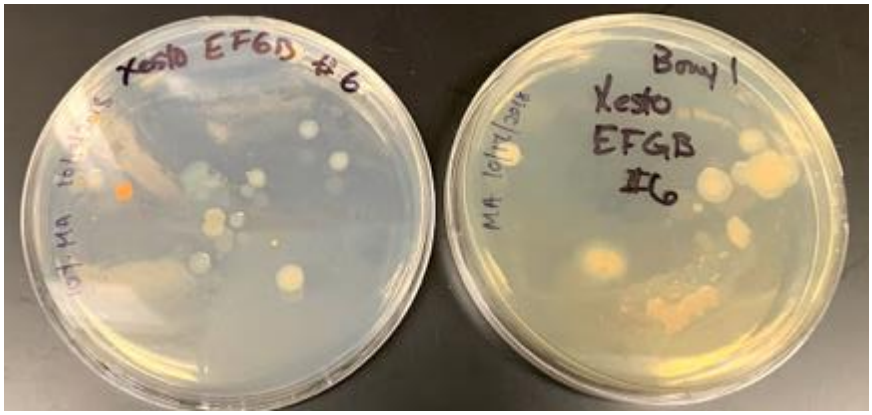
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EFGB
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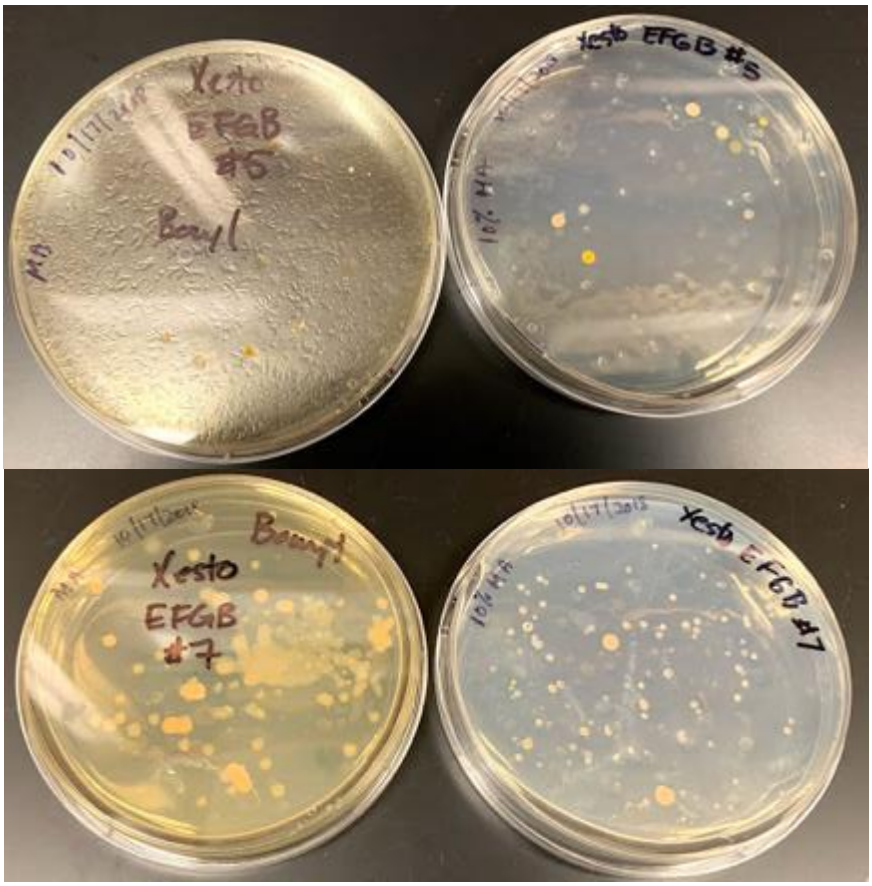
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#5
Bcayl
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Bcayl
Xesto
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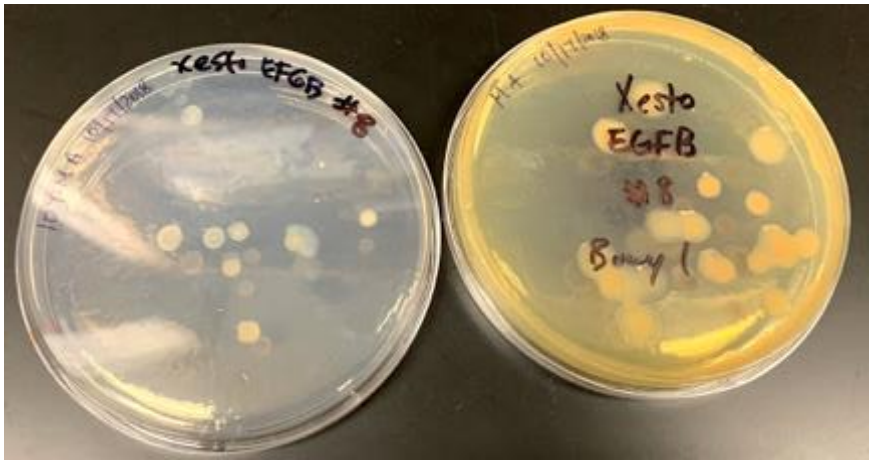
#7
Xesb EFGB #7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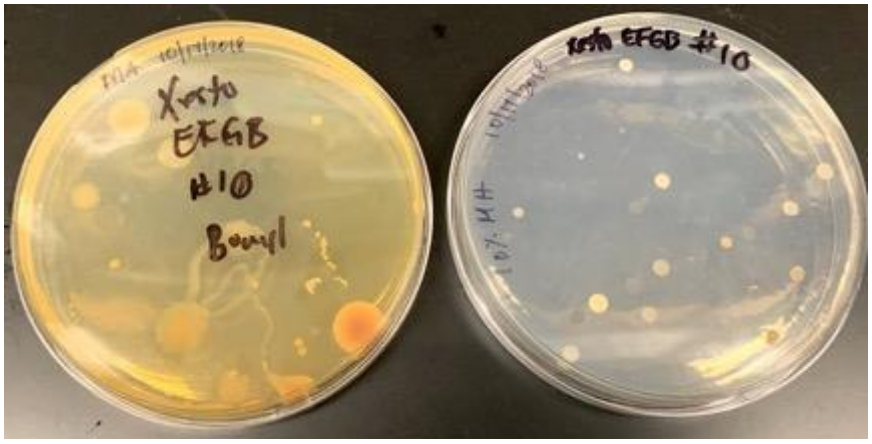
Xesto
EGFB
#8
Bowyl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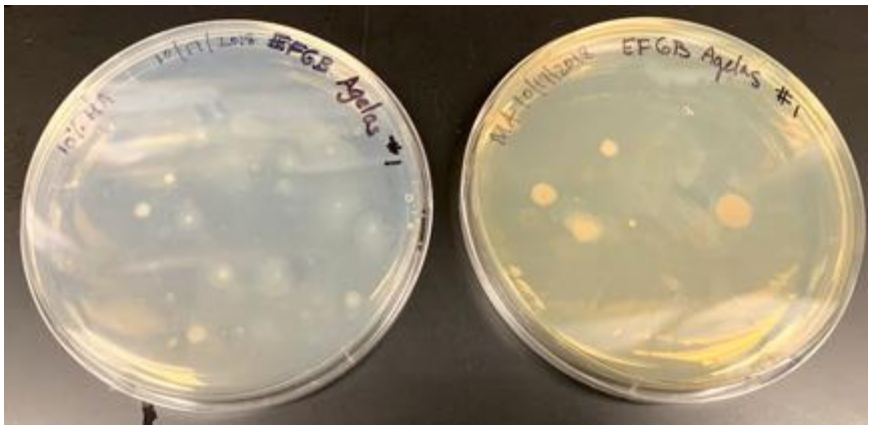
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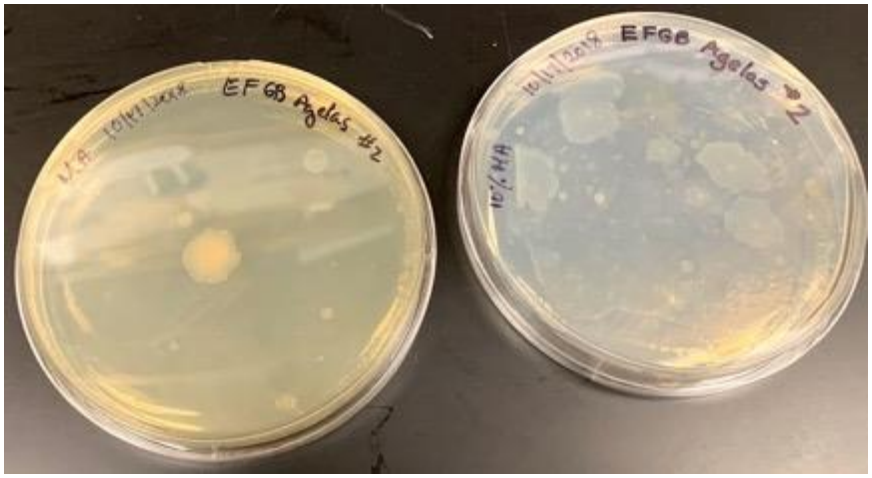
EFGB Agelas #1


EFGB #9
Xesto
EFGB
#9
Bowl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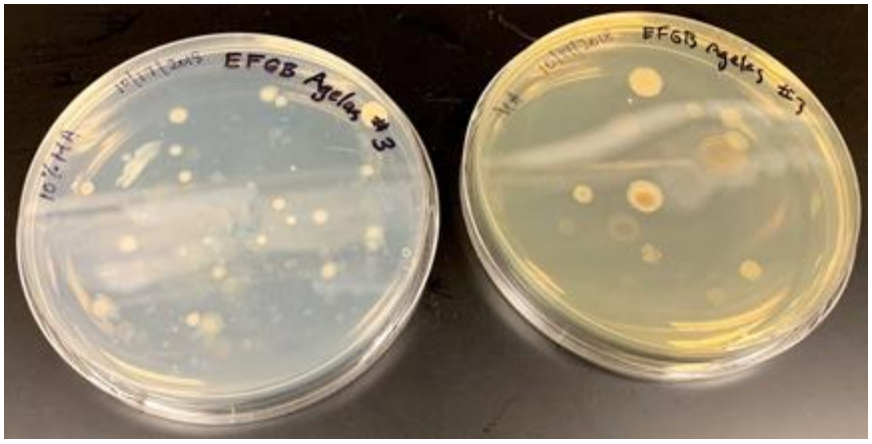
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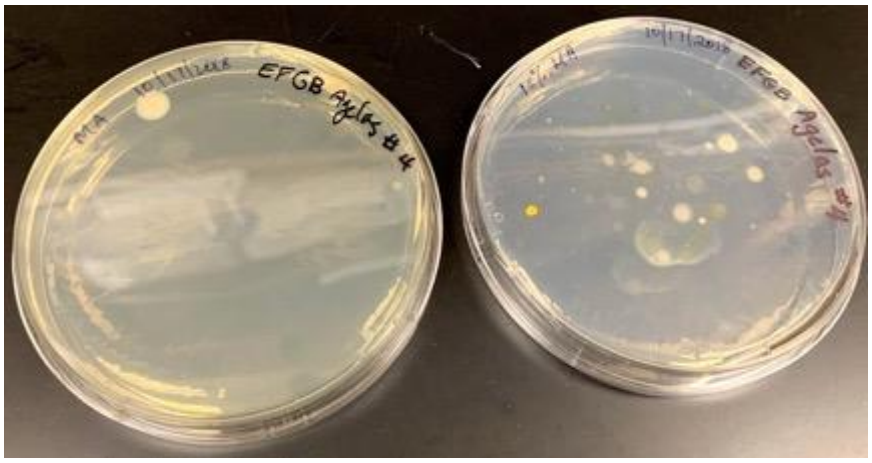
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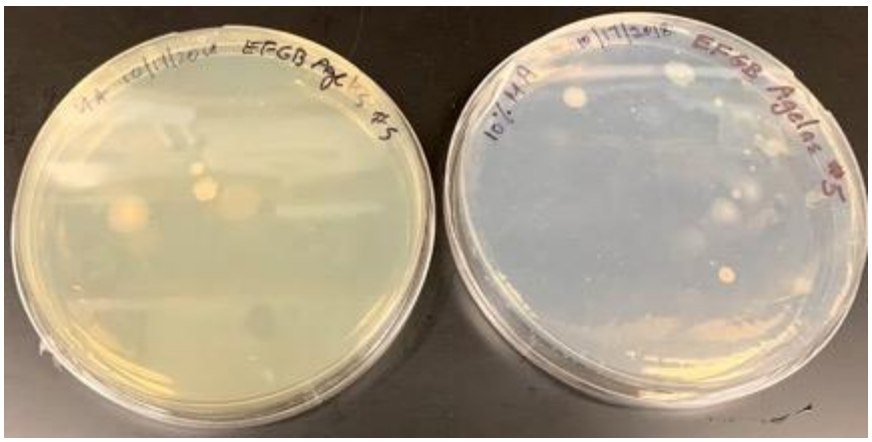
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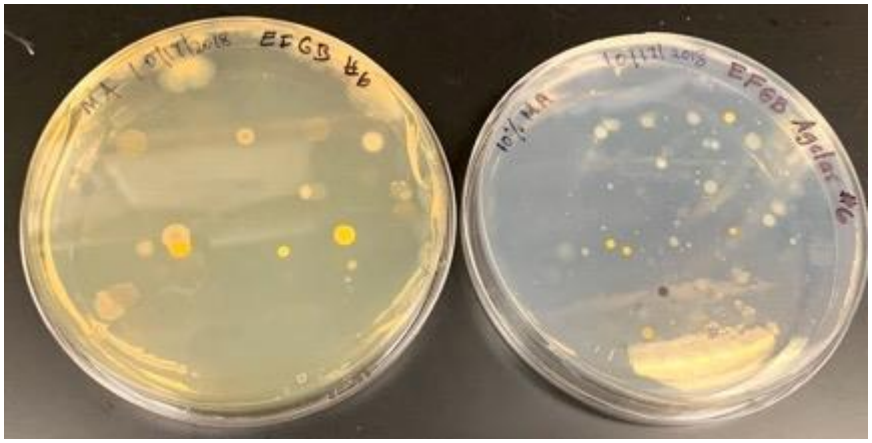
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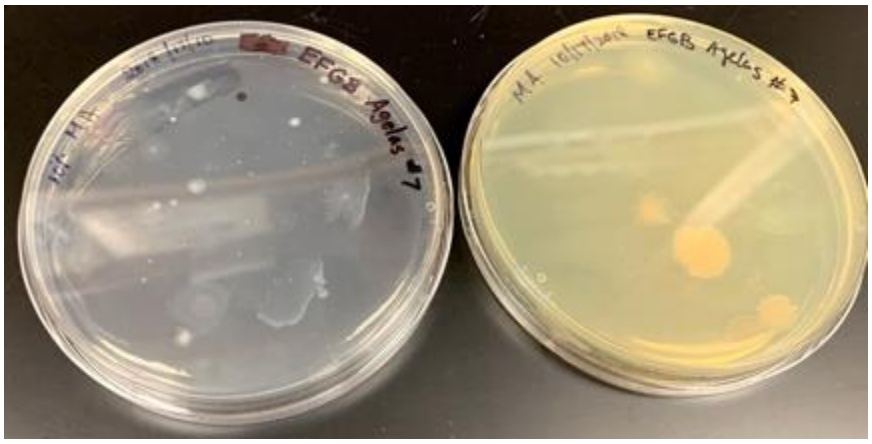
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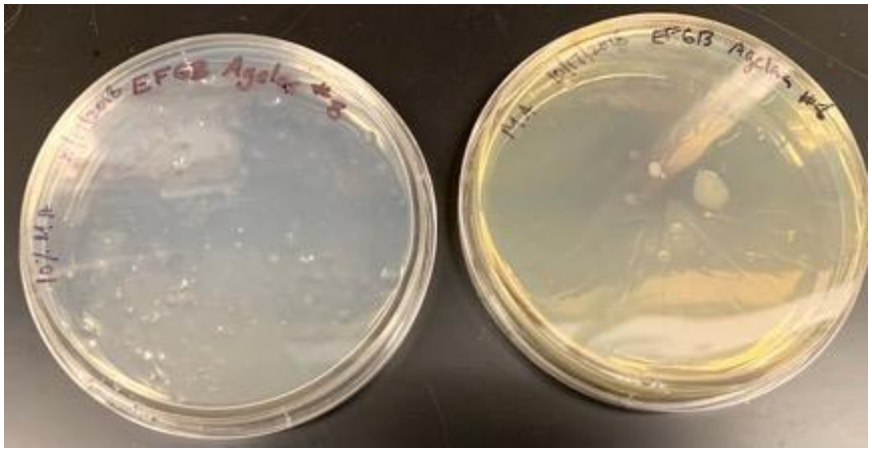
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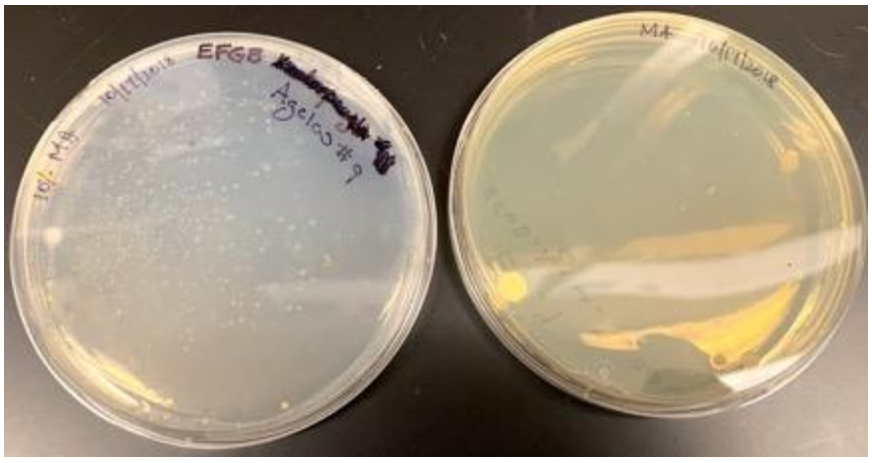
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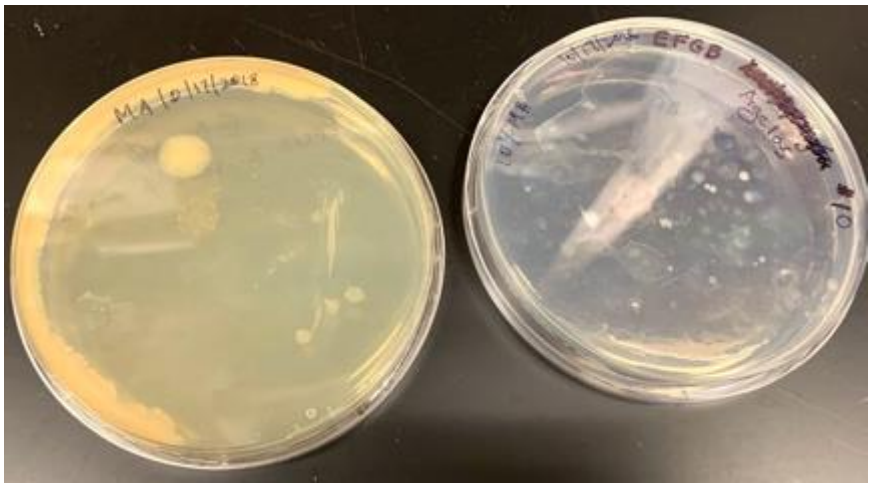

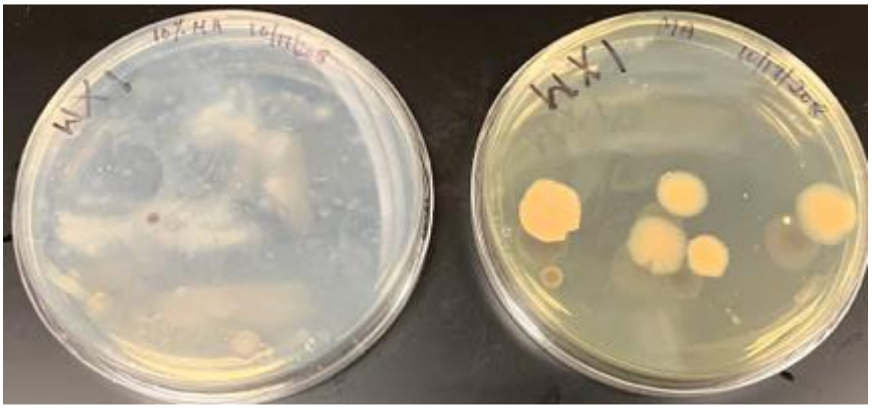

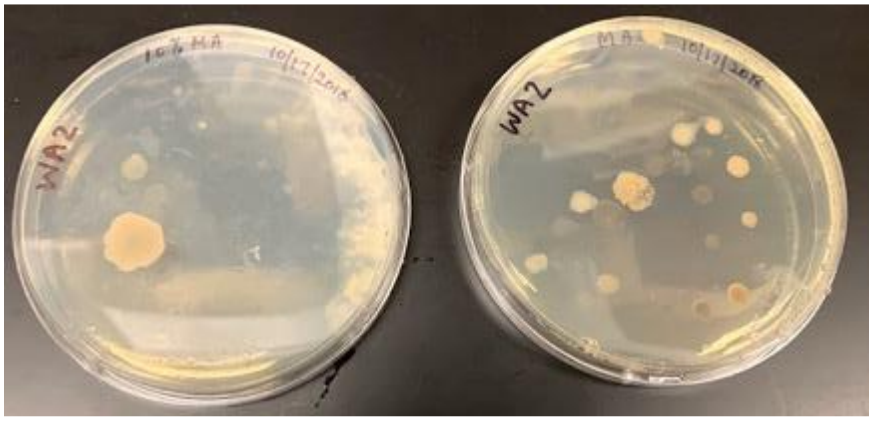

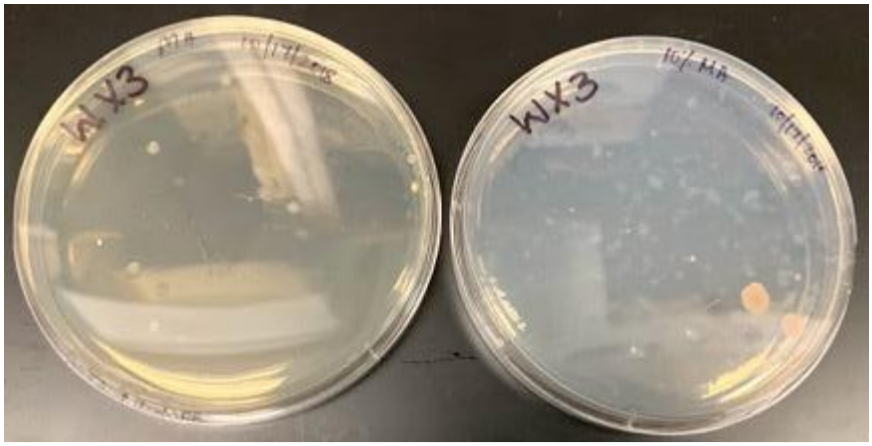
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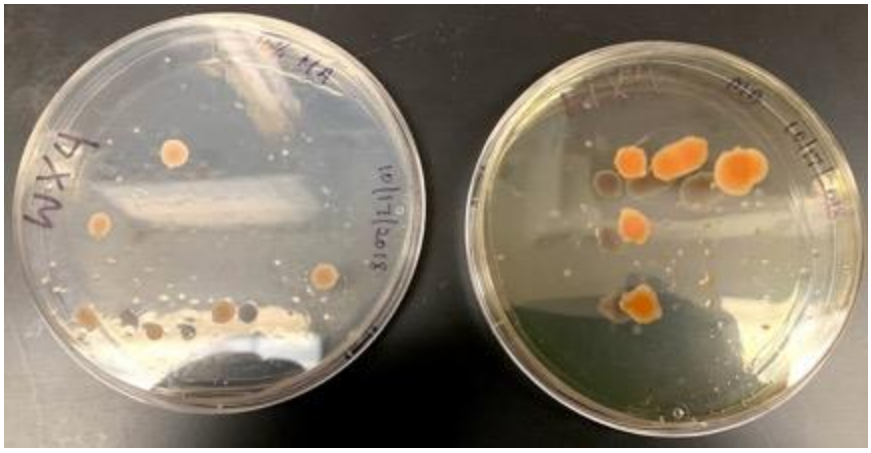
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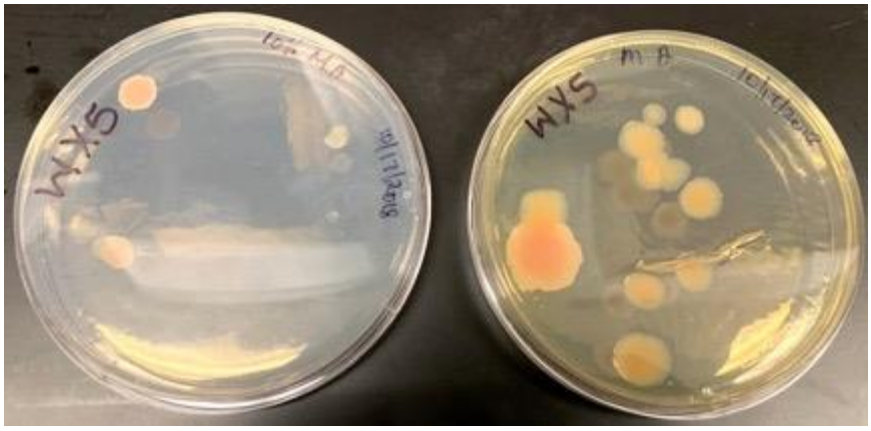
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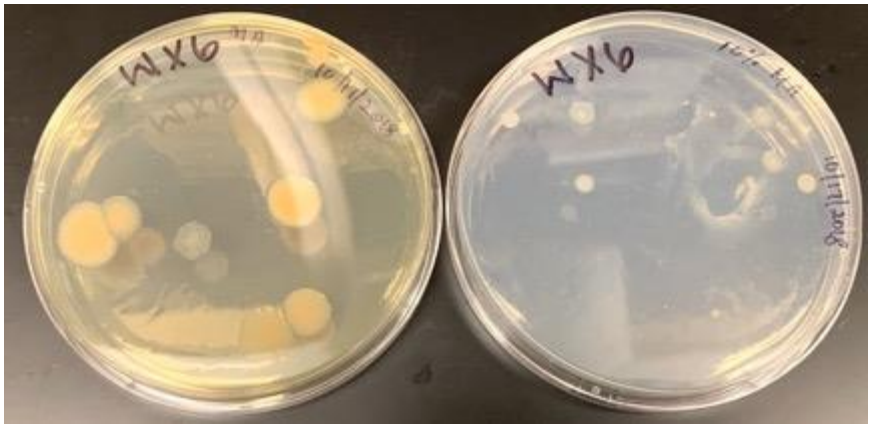
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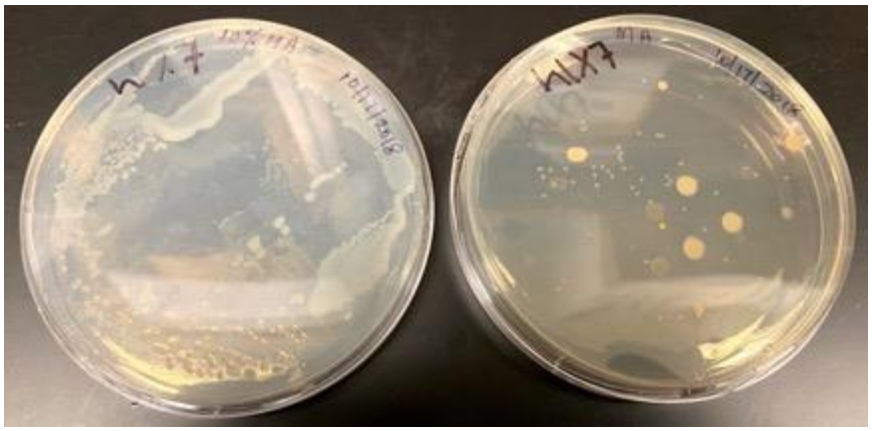
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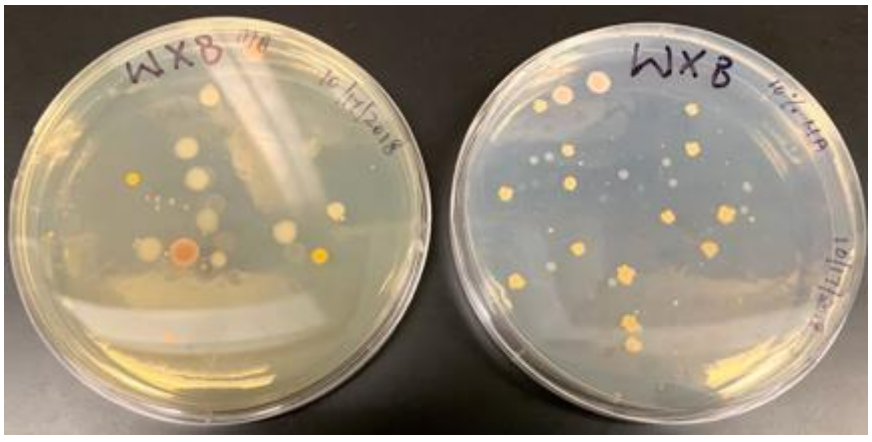
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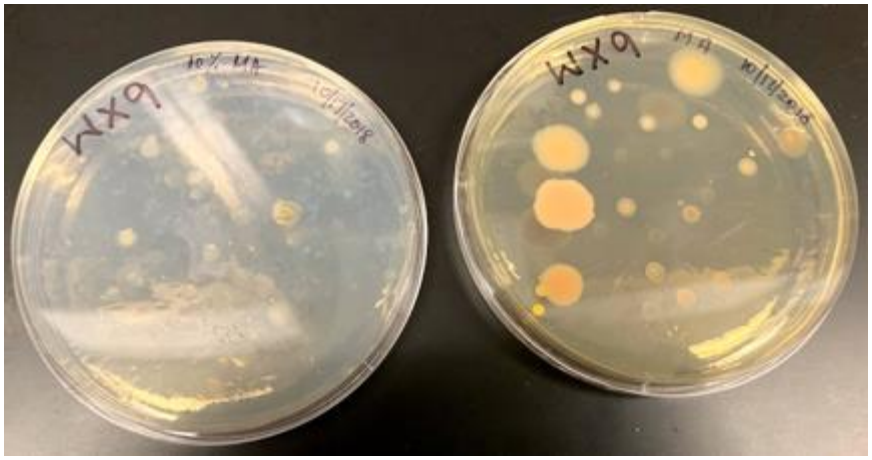
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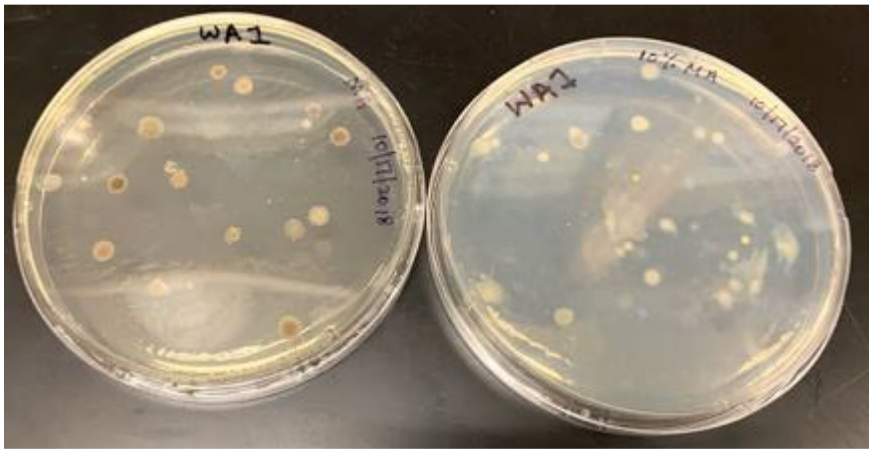
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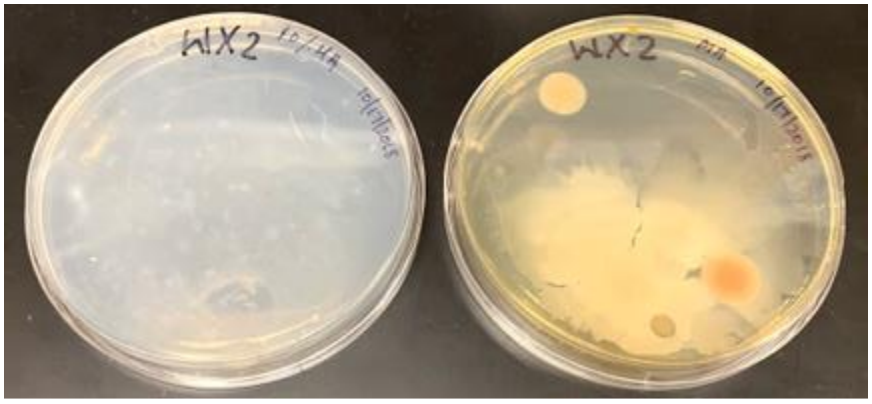
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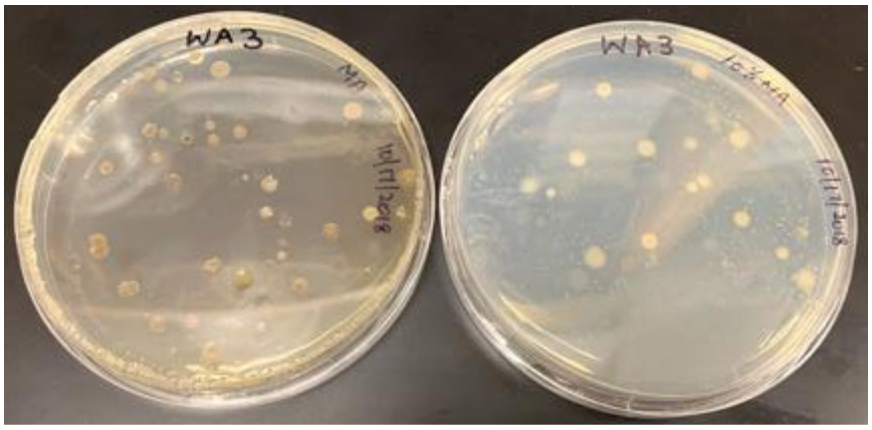
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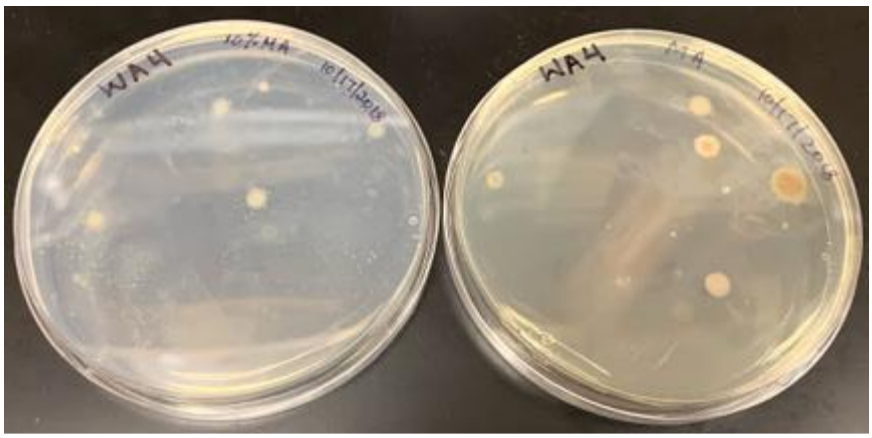
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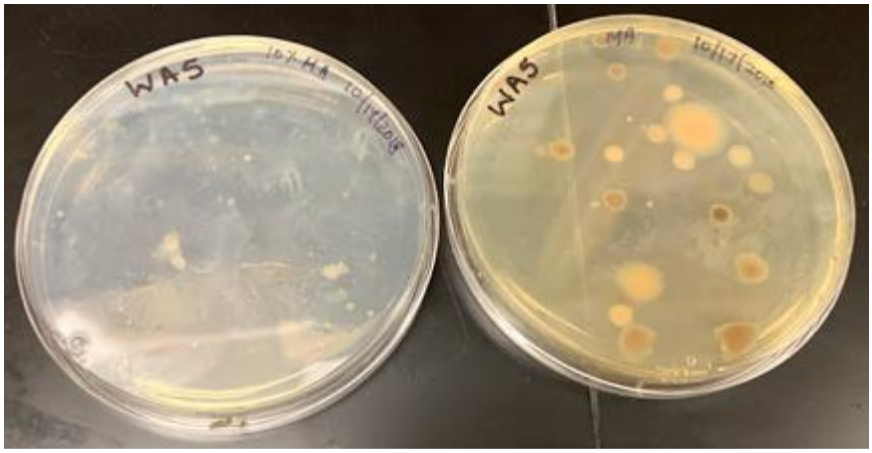
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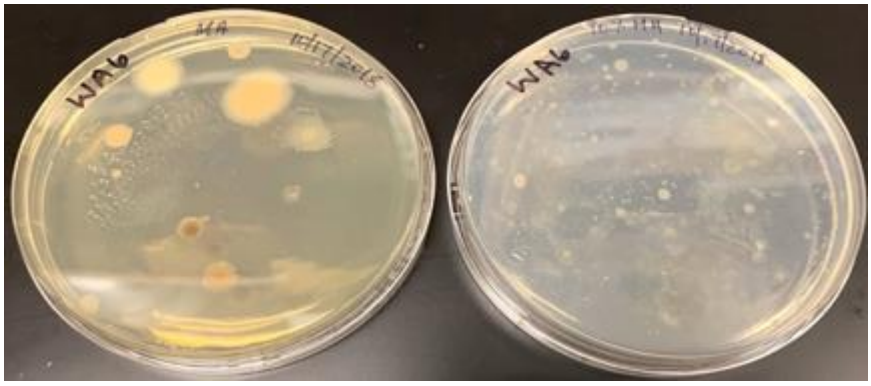
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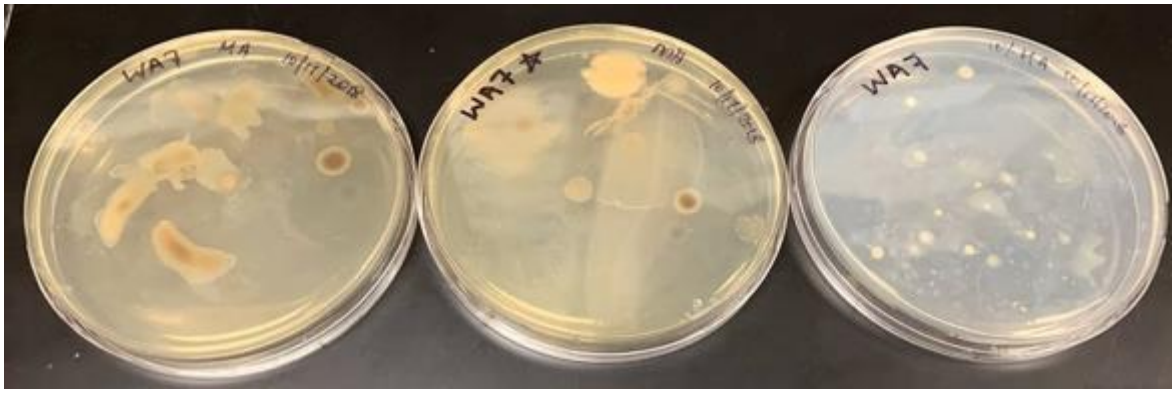
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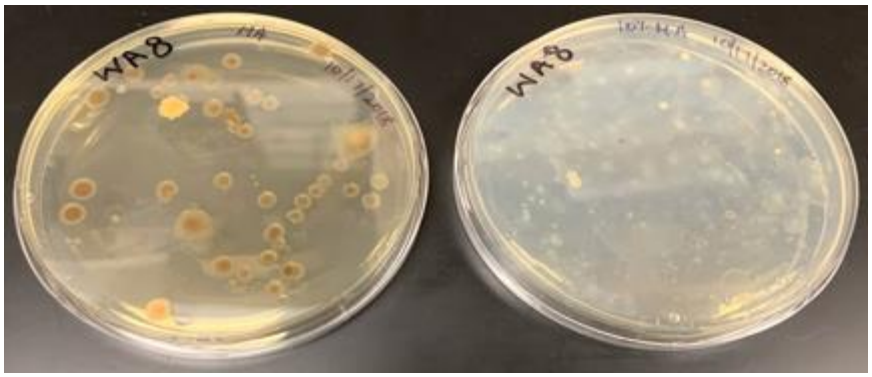
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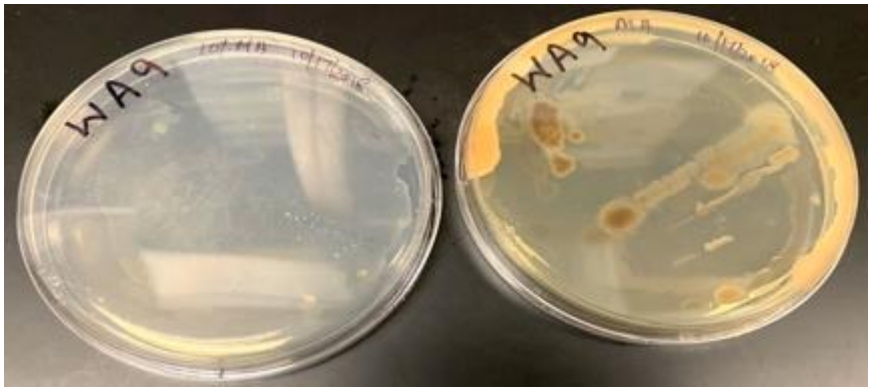
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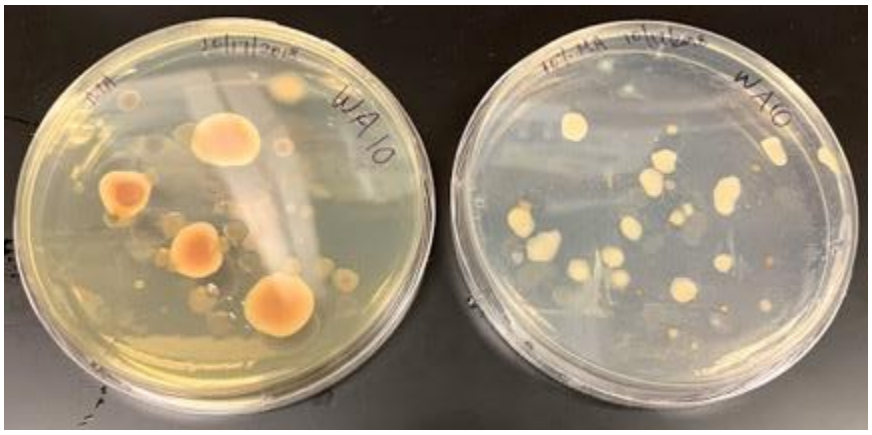
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APPENDIX C:

BACTERIA IDENTIFICATION

Bacteria Identification. 16s rDNA sequences were used to determine the identity of the bacteria using NCBI Blast. Samples with %ID under 98% were identified to class, samples with %ID above 98% were identified to species when possible.

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMEB001-18	MA	XHE81L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	1.00E-156	100.00%	MN577288.1	Unknown
XMEB003-18	MA	XHE83L	No Sequence					
XMEB005-18	MA	XHE83L	No Sequence					
XMEB006-18	MA	XHE84L	No Sequence					
XMEB007-18	MA	XHE84L	No Sequence					
XMEB009-18	MA	XHE84L	g-proteobacteria	Pseudomonas stutzeri strain NNA20	2.00E-128	100.00%	MT379492.1	Mangrove sediment
XMEB010-18	MA	XHE84L	No Sequence					
XMEB012-18	MA	XHE85L	No Sequence					
XMEB013-18	MA	XHE86L	g-proteobacteria	Psychrobacter marincola strain QS123	6.00E-144	98.32%	MK439597.1	Unknown
XMEB014-18	MA	XHE86L	No Sequence					
XMEB015-18	MA	XHE86L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMEB016-18	MA	XHE86L	g-proteobacteria	Psychrobacter marincola strain QS123	2.00E-149	99.33%	MK439597.1	Unknown
XMEB017-18	MA	XHE87L	Not reliable identification					
XMEB018-18	MA	XHE87L	Not reliable identification					
XMEB019-18	MA	XHE87L	Not reliable identification					
XMEB020-18	MA	XHE87L	Not reliable identification					
XMEB021-18	MA	XHE88L	No Sequence					
XMEB022-18	MA	XHE88L	g-proteobacteria	Psychrobacter submarinus strain ACBC159	4.00E-79	95.30%	MK214728.1	Southern ocean
XMEB023-18	MA	XHE88L	Not reliable identification					
XMEB024-18	MA	XHE88L	Not reliable identification					
XMEB025-18	MA	XHE89L	Not reliable identification					
XMEB026-18	MA	XHE89L	Not reliable identification					
XMEB027-18	MA	XHE89L	Not reliable identification					
XMEB028-18	MA	XHE89L	Not reliable identification					
XMEB029-18	MA	XHE810L	g-proteobacteria	Psychrobacter sp. GN87	2.00E-61	97.28%	KJ719402.1	seawater
XMEB030-18	MA	XHE810L	Not reliable identification					
XMEB031-18	MA	XHE810L	Not reliable identification					
XMEB032-18	MA	XHE810L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMEB033-18	10% MA	XHE81L	Not reliable identification					
XMEB034-18	10% MA	XHE81L	g-proteobacteria	Endozoicomonas acroporae strain Acr-14	3.00E-152	99.67%	NR_158127.1	Acropora coral
XMEB035-18	10% MA	XHE812L	Not reliable identification					
XMEB043-18	10% MA	XHE812L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-150	100.00%	MT187959.1	scleractinian coral
XMEB044-18	10% MA	XHE81L	g-proteobacteria	Pseudoalteromonas sp. 1006	2.00E-144	98.98%	AM110952.1	deep sea sediment
XMEB045-18	10% MA	XHE812L	Not reliable identification					
XMEB036-18	10% MA	XHE812L	Not reliable identification					
XMEB037-18	10% MA	XHE812L	Not reliable identification					
XMEB038-18	10% MA	XHE83L	No Sequence					
XMEB039-18	10% MA	XHE83L	Not reliable identification					
XMEB049-18	10% MA	XHE84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	4.00E-156	100.00%	MT187959.1	scleractinian coral
XMEB040-18	10% MA	XHE84L	Not reliable identification					
XMEB041-18	10% MA	XHE84L	Not reliable identification					
XMEB043-18	10% MA	XHE84L	Not reliable identification					
XMEB050-18	10% MA	XHE84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	6.00E-154	100.00%	MT187959.1	scleractinian coral
XMEB051-18	10% MA	XHE85L	a-proteobacteria	Erythrobacter citreus strain HYSJ108	2.00E-149	100.00%	MN746238.1	dinoflagellate bloom

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMEB052-18	10% MA	XHE85L	Not reliable identification					
XMEB055-18	10% MA	XHE85L	Not reliable identification					
XMEB056-18	10% MA	XHE86L	Not reliable identification					
XMEB057-18	10% MA	XHE86L	Not reliable identification					
XMEB058-18	10% MA	XHE86L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	6.00E-149	100.00%	MT187959.1	scleractinian coral
XMEB059-18	10% MA	XHE86L	Not reliable identification					
XMEB060-18	10% MA	XHE86L	a-proteobacteria	Erythrobacter sp. strain JBTF-M22	2.00E-149	99.33%	MN872419.1	tidal flat sediment
XMEB061-18	10% MA	XHE86L	Not reliable identification					
XMEB062-18	10% MA	XHE86L	Not reliable identification					
XMEB063-18	10% MA	XHE87L	Not reliable identification					
XMEB064-18	10% MA	XHE87L	Not reliable identification					
XMEB065-18	10% MA	XHE87L	Not reliable identification					
XMEB066-18	10% MA	XHE87L	Not reliable identification					
XMEB067-18	10% MA	XHE87L	Not reliable identification					
XMEB068-18	10% MA	XHE88L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	2.00E-149	99.66%	MT187959.1	scleractinian coral
XMEB069-18	10% MA	XHE88L	Not reliable identification					
XMEB070-18	10% MA	XHE88L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMEB071-18	10% MA	XHE88L	Not reliable identification					
XMEB072-18	10% MA	XHE88L	g-proteobacteria	Alteromonas macleodii strain BC72	2.00E-149	99.33%	MT325885.1	marine bacteria
XMEB073-18	10% MA	XHE89L	Not reliable identification					
XMEB074-18	10% MA	XHE89L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-88	98.44%	MT187959.1	scleractinian coral
XMEB075-18	10% MA	XHE89L	Not reliable identification					
XMEB076-18	10% MA	XHE89L	Not reliable identification					
XMEB078-18	10% MA	XHE810L	Not reliable identification					
XMEB079-18	10% MA	XHE810L	Not reliable identification					
XMEB080-18	10% MA	XHE810L	Not reliable identification					
XMEB081-18	10% MA	XHE810L	Not reliable identification					
XMEB082-18	10% MA	XHE810L	Not reliable identification					
XMEB083-18	MA	XHE810L	Not reliable identification					
ACEB084-18	MA	AHE81L	g-proteobacteria	Microbulbifer sp. strain RAR_M1_41	1.00E-151	100.00%	MT229085.1	mangrove plant
ACEB085-18	MA	AHE81L	Not reliable identification					
ACEB086-18	MA	AHE81L	Not reliable identification					
ACEB087-18	MA	AHE81L	Not reliable identification					
ACEB088-18	MA	AHE81L	g-proteobacteria	Microbulbifer sp. strain RAR_M1_41	1.00E-151	100.00%	MT229085.1	mangrove plant

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACEB089-18	MA	AHE82L	g-proteobacteria	Endozoicomonas montiporae CL-33	1.00E-146	98.99%	CP013251.1	encrusting pore coral
ACEB090-18	MA	AHE82L	Not reliable identification					
ACEB091-18	MA	AHE82L	Not reliable identification					
ACEB092-18	MA	AHE82L	Not reliable identification					
ACEB093-18	MA	AHE82L	Not reliable identification					
ACEB094-18	MA	AHE82L	Not reliable identification					
ACEB095-18	MA	AHE82L	Not reliable identification					
ACEB096-18	MA	AHE83L	Not reliable identification					
ACEB097-18	MA	AHE83L	Not reliable identification					
ACEB098-18	MA	AHE83L	Not reliable identification					
ACEB099-18	MA	AHE83L	Not reliable identification					
ACEB100-18	MA	AHE83L	Not reliable identification					
ACEB101-18	MA	AHE83L	Not reliable identification					
ACEB102-18	MA	AHE83L	Not reliable identification					
ACEB103-18	MA	AHE84L	Not reliable identification					
ACEB104-18	MA	AHE84L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACEB105-18	MA	AHE85L	Not reliable identification					
ACEB106-18	MA	AHE85L	Not reliable identification					
ACEB107-18	MA	AHE85L	g-proteobacteria	Endozoicomonas sp. SF204	4.00E-161	99.07%	KM360464.1	coral mucus
ACEB108-18	MA	AHE85L	Not reliable identification					
ACEB110-18	MA	AHE86L	g-proteobacteria	Endozoicomonas sp. SF204	4.00E-161	99.07%	KM360464.1	coral mucus
ACEB111-18	MA	AHE86L	a-proteobacteria	Ruegeria atlantica strain ROA004	6.00E-144	98.97%	MT072134.1	Deep sea sediment
ACEB112-18	MA	AHE86L	Not reliable identification					
ACEB113-18	MA	AHE86L	g-proteobacteria	Shewanella sp. JZ11IS74	2.00E-153	98.41%	KC429938.1	marine sponges
ACEB114-18	MA	AHE86L	Not reliable identification					
ACEB115-18	MA	AHE86L	Not reliable identification					
ACEB116-18	MA	AHE86L	Not reliable identification					
ACEB117-18	MA	AHE86L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	3.00E-157	98.75%	MH201037.1	sponge
ACEB118-18	MA	AHE86L	Not reliable identification					
ACEB119-18	MA	AHE87L	g-proteobacteria	Microbulbifer sp. strain Aga-ANL-00-2	6.00E-159	98.76%	MK453492.1	macroalgae
ACEB120-18	MA	AHE87L	a-proteobacteria	Pseudovibrio japonicus strain QS305	2.00E-153	98.72%	MK439596.1	Unknown
ACEB121-18	MA	AHE87L	g-proteobacteria	Microbulbifer sp. C10-1	1.00E-160	99.07%	LC498625.1	coral
ACEB123-18	MA	AHE88L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACEB124-18	MA	AHE88L	g-proteobacteria	Endozoicomonas sp. SF204	1.00E-155	99.04%	KM360464.1	coral mucus
ACEB125-18	MA	AHE88L	g-proteobacteria	Vibrio sp. LMF	2.00E-159	99.68%	KU560496.1	Lobophora monticola
ACEB126-18	MA	AHE88L	Not reliable identification					
ACEB127-18	MA	AHE88L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK214693.1	Southern ocean
ACEB128-18	MA	AHE89L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean
ACEB129-18	MA	AHE89L	g-proteobacteria	Psychrobacter sp. strain P151-L015a	6.00E-154	99.67%	MN043901.1	sediment
ACEB131-18	MA	AHE810L	Not reliable identification					
ACEB132-18	MA	AHE810L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean
ACEB133-18	MA	AHE810L	Not reliable identification					
ACEB134-18	MA	AHE810L	Not reliable identification					
ACEB135-18	MA	AHE810L	Not reliable identification					
ACEB136-18	MA	AHE810L	g-proteobacteria	Microbulbifer sp. THAF38	4.00E-156	98.15%	CP045369.1	Surface of a microplastic
ACEB137-18	10% MA	AHE81L	a-proteobacteria	Pseudovibrio japonicus strain QS305	0.0	99.04%	MK439596.1	Unknown
ACEB138-18	10% MA	AHE81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-155	99.67%	MH201037.1	sponge
ACEB139-18	10% MA	AHE81L	a-proteobacteria	Pseudovibrio sp. FO-BEG1	3.00E-157	98.75%	KX418559.1	marine sponge
ACEB140-18	10% MA	AHE81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	6.00E-154	98.13%	MH201037.1	sponge

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACEB141-18	10% MA	AHE81L	Not reliable identification					
ACEB142-18	10% MA	AHE82L	Not reliable identification					
ACEB143-18	10% MA	AHE82L	a-proteobacteria	Pseudovibrio sp. FO-BEG1	6.00E-159	99.06%	KX418559.1	marine sponge
ACEB144-18	10% MA	AHE82L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	0.0	98.12%	MH201037.1	sponge
ACEB145-18	10% MA	AHE82L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-158	99.06%	MH201037.1	sponge
ACEB146-18	10% MA	AHE82L	a-proteobacteria	Pseudovibrio sp. strain RAR_M1_23	0.0	99.03%	MT229094.1	mangrove plant
ACEB147-18	10% MA	AHE83L	Not reliable identification					
ACEB148-18	10% MA	AHE83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-158	99.06%	MH201037.1	sponge
ACEB149-18	10% MA	AHE83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	0.0	99.04%	MH201037.1	sponge
ACEB150-18	10% MA	AHE83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-156	99.36%	MH201037.1	sponge
ACEB151-18	10% MA	AHE83L	Not reliable identification					
ACEB152-18	10% MA	AHE84L	Not reliable identification					
ACEB153-18	10% MA	AHE84L	Not reliable identification					
ACEB154-18	10% MA	AHE84L	Not reliable identification					
ACEB155-18	10% MA	AHE84L	Not reliable identification					
ACEB156-18	10% MA	AHE84L	Not reliable identification					
ACEB157-18	10% MA	AHE84L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACEB158-18	10% MA	AHE84L	Not reliable identification					
ACEB159-18	10% MA	AHE85L	Not reliable identification					
ACEB160-18	10% MA	AHE85L	Not reliable identification					
ACEB161-18	10% MA	AHE85L	Not reliable identification					
ACEB162-18	10% MA	AHE85L	Not reliable identification					
ACEB163-18	10% MA	AHE85L	Not reliable identification					
ACEB164-18	10% MA	AHE86L	Not reliable identification					
ACEB165-18	10% MA	AHE86L	Not reliable identification					
ACEB166-18	10% MA	AHE86L	Not reliable identification					
ACEB167-18	10% MA	AHE86L	Not reliable identification					
ACEB168-18	10% MA	AHE86L	Not reliable identification					
ACEB169-18	10% MA	AHE86L	Not reliable identification					
ACEB170-18	10% MA	AHE86L	Not reliable identification					
ACEB171-18	10% MA	AHE87L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean
ACEB172-18	10% MA	AHE87L	Not reliable identification					
ACEB173-18	10% MA	AHE87L	Not reliable identification					
ACEB174-18	10% MA	AHE88L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACEB175-18	10% MA	AHE88L	Not reliable identification					
ACEB176-18	10% MA	AHE88L	Not reliable identification					
ACEB177-18	10% MA	AHE88L	Not reliable identification					
ACEB178-18	10% MA	AHE88L	Not reliable identification					
ACEB179-18	10% MA	AHE89L	Not reliable identification					
ACEB180-18	10% MA	AHE89L	Not reliable identification					
ACEB181-18	10% MA	AHE89L	Not reliable identification					
ACEB182-18	10% MA	AHE89L	a-proteobacteria	Ruegeria atlantica strain ROA004	1.00E-140	99.64%	MT072134.1	deep sea sediment
ACEB183-18	10% MA	AHE89L	Not reliable identification					
ACEB184-18	10% MA	AHE89L	Not reliable identification					
ACEB185-18	10% MA	AHE89L	Not reliable identification					
ACEB186-18	10% MA	AHE810L	Not reliable identification					
ACEB187-18	10% MA	AHE810L	Not reliable identification					
ACEB188-18	10% MA	AHE810L	Not reliable identification					
XMWB189-18	10% MA	XHW81L	Not reliable identification					
XMWB190-18	MA	XHW81L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean
XMWB191-18	MA	XHW81L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMWB192-18	MA	XHW81L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-155	99.35%	MT187959.1	scleractinian coral
XMWB193-18	MA	XHW82L	Not reliable identification					
XMWB194-18	MA	XHW82L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-156	100.00%	MT187959.1	scleractinian coral
XMWB195-18	MA	XHW82L	Not reliable identification					
XMWB196-18	MA	XHW82L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean
XMWB197-18	MA	XHW82L	g-proteobacteria	Psychrobacter marincola strain ACBC157	5.00E-155	99.67%	MK439597.1	Southern ocean
XMWB198-18	MA	XHW83L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	2.00E-154	100.00%	MT187959.1	scleractinian coral
XMWB199-18	MA	XHW83L	Not reliable identification					
XMWB200-18	MA	XHW84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	8.00E-158	99.36%	MT187959.1	scleractinian coral
XMWB201-18	MA	XHW84L	Not reliable identification					
XMWB202-18	MA	XHW84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	3.00E-157	99.68%	MT187959.1	scleractinian coral
XMWB203-18	MA	XHW84L	Not reliable identification					
XMWB204-18	MA	XHW85L	Not reliable identification					
XMWB205-18	MA	XHW85L	Not reliable identification					
XMWB206-18	MA	XHW85L	g-proteobacteria	Alteromonas macleodii strain BC72	3.00E-151	100.00%	MT325885.1	marine sediment
XMWB210-18	MA	XHW86L	Not reliable identification					
XMWB211-18	MA	XHW87L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMWB212-18	MA	XHW87L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	4.00E-140	100.00%	MT187959.1	scleractinian coral
XMWB213-18	MA	XHW87L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	3.00E-157	100.00%	MT187959.1	scleractinian coral
XMWB214-18	MA	XHW88L	g-proteobacteria	Pseudomonas stutzeri strain NNA20	3.00E-152	100.00%	MT379492.1	Mangrove sediment
XMWB215-18	MA	XHW88L	g-proteobacteria	Pseudomonas stutzeri strain NNA20	2.00E-128	100.00%	MT379492.1	Mangrove sediment
XMWB216-18	MA	XHW88L	g-proteobacteria	Pseudomonas sp. strain INP3 16S	5.00E-155	99.67%	MT197174.1	seawater
XMWB217-18	MA	XHW88L	g-proteobacteria	Pseudomonas xinjiangensis strain Y59	3.00E-157	100.00%	KU601273.1	soil
XMWB218-18	MA	XHW89L	Not reliable identification					
XMWB219-18	MA	XHW89L	g-proteobacteria	Acinetobacter radioresistens strain SRSU4	1.00E-156	100.00%	MT258988.1	Labeo rohita
XMWB220-18	MA	XHW89L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-153	99.67%	MH201037.1	sponge
XMWB221-18	MA	XHW89L	g-proteobacteria	Gammaproteobacteria bacterium AaS03	6.00E-144	100.00%	KP412854.1	marine sponge
XMWB222-18	MA	XHW810L	Not reliable identification					
XMWB223-18	MA	XHW810L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-150	100.00%	MT187959.1	scleractinian coral
XMWB224-18	MA	XHW810L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	3.00E-151	100.00%	MT187959.1	scleractinian coral
XMWB225-18	MA	XHW81L	Not reliable identification					
XMWB226-18	10% MA	XHW81L	Not reliable identification					
XMWB228-18	10% MA	XHW82L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMWB231-18	10% MA	XHW83L	Not reliable identification					
XMWB232-18	10% MA	XHW83L	Not reliable identification					
XMWB233-18	10% MA	XHW83L	Not reliable identification					
XMWB234-18	10% MA	XHW84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-156	100.00%	MT187959.1	scleractinian coral
XMWB235-18	10% MA	XHW84L	Not reliable identification					
XMWB236-18	10% MA	XHW84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	3.00E-151	100.00%	MT187959.1	scleractinian coral
XMWB237-18	10% MA	XHW85L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0059	1.00E-156	100.00%	MT187910.1	scleractinian coral
XMWB238-18	10% MA	XHW85L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	1.00E-155	100.00%	MT187959.1	scleractinian coral
XMWB239-18	10% MA	XHW85L	Not reliable identification					
XMWB240-18	10% MA	XHW86L	Not reliable identification					
XMWB241-18	10% MA	XHW86L	Not reliable identification					
XMWB242-18	10% MA	XHW86L	Not reliable identification					
XMWB243-18	10% MA	XHW87L	Not reliable identification					
XMWB244-18	10% MA	XHW87L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	2.00E-154	99.67%	MT187959.1	scleractinian coral
XMWB245-18	10% MA	XHW87L	Not reliable identification					
XMWB246-18	10% MA	XHW88L	g-proteobacteria	Psychrobacter marincola strain ACBC157	2.00E-153	99.67%	MK439597.1	Southern ocean
XMWB247-18	10% MA	XHW88L	g-proteobacteria	Acinetobacter radioresistens strain SRSU4	1.00E-156	100.00%	MT258988.1	Labeo rohita

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
XMWB248-18	10% MA	XHW88L	g-proteobacteria	Vibrio fortis strain DS1807-4	6.00E-149	99.33%	MT269591.1	sea water
XMWB249-18	10% MA	XHW88L	Not reliable identification					
XMWB250-18	10% MA	XHW89L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	6.00E-154	99.35%	MH201037.1	sponge
XMWB251-18	10% MA	XHW89L	Not reliable identification					
XMWB252-18	10% MA	XHW89L	g-proteobacteria	Psychrobacter sp. ch47	3.00E-157	100.00%	LC379513.1	bottom sediments
XMWB253-18	10% MA	XHW89L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	8.00E-153	100.00%	MT187959.1	
XMWB254-18	10% MA	XHW89L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	4.00E-151	99.34%	MH201037.1	sponge
XMWB255-18	10% MA	XHW810L	Not reliable identification					
XMWB256-18	10% MA	XHW810L	Not reliable identification					
XMWB257-18	10% MA	XHW810L	No Sequence					
XMWB258-18	10% MA	XHW810L	Not reliable identification					
XMWB259-18	10% MA	XHW810L	Not reliable identification					
ACWB260-18	MA	AHW81L	Not reliable identification					
ACWB261-18	MA	AHW81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-155	100.00%	MH201037.1	sponge
ACWB262-18	MA	AHW81L	Not reliable identification					
ACWB263-18	MA	AHW81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps18	1.00E-151	99.34%	MH201038.1	sponge
ACWB264-18	MA	AHW81L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB265-18	MA	AHW81L	Not reliable identification					
ACWB266-18	MA	AHW81L	Not reliable identification					
ACWB267-18	MA	AHW81L	Not reliable identification					
ACWB268-18	MA	AHW81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	3.00E-152	99.67%	MH201037.1	sponge
ACWB269-18	MA	AHW81L	Not reliable identification					
ACWB270-18	MA	AHW81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-155	99.67%	MH201037.1	sponge
ACWB271-18	MA	AHW81L	Not reliable identification					
ACWB272-18	MA	AHW81L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-151	99.67%	MH201037.1	sponge
ACWB273-18	MA	AHW81L	g-proteobacteria	Acinetobacter radioresistens strain SRSU4	4.00E-145	100.00%	MT258988.1	Labeo rohita
ACWB274-18	MA	AHW82L	g-proteobacteria	Microbulbifer sp. THAF38	1.00E-151	100.00%	CP045369.1	Surface of a microplastic particle
ACWB275-18	MA	AHW82L	Not reliable identification					
ACWB276-18	MA	AHW82L	Not reliable identification					
ACWB277-18	MA	AHW82L	a-proteobacteria	Pseudovibrio japonicus strain QS305	1.00E-151	99.34%	MK439596.1	Unknown
ACWB278-18	MA	AHW82L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	6.00E-154	100.00%	MH201037.1	sponge
ACWB279-18	MA	AHW82L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	6.00E-144	100.00%	MH201037.1	sponge
ACWB280-18	MA	AHW82L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB281-18	MA	AHW83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-154	99.35%	MH201037.1	sponge
ACWB282-18	MA	AHW83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-155	100.00%	MH201037.1	sponge
ACWB283-18	MA	AHW83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	8.00E-153	99.67%	MH201037.1	sponge
ACWB284-18	MA	AHW83L	Not reliable identification					
ACWB285-18	MA	AHW83L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB286-18	MA	AHW83L	a-proteobacteria	Pseudovibrio japonicus strain QS305	1.00E-151	99.34%	MK439596.1	Unknown
ACWB287-18	MA	AHW83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-153	99.67%	MH201037.1	sponge
ACWB288-18	MA	AHW84L	Not reliable identification					
ACWB289-18	MA	AHW84L	g-proteobacteria	Microbulbifer sp. strain RAR_M1_41	5.00E-155	99.67%	MT229085.1	mangrove plant
ACWB290-18	MA	AHW84L	Not reliable identification					
ACWB291-18	MA	AHW84L	g-proteobacteria	Microbulbifer sp. THAF38	4.00E-156	98.15%	CP045369.1	Surface of a microplastic particle
ACWB292-18	MA	AHW84L	g-proteobacteria	Microbulbifer sp. strain RAR_M1_41	3.00E-151	100.00%	MT229085.1	mangrove plant
ACWB293-18	MA	AHW84L	Not reliable identification					
ACWB295-18	MA	AHW85L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	2.00E-154	99.67%	MT187959.1	scleractinian coral
ACWB296-18	MA	AHW85L	g-proteobacteria	Microbulbifer sp. strain RAR_M1_41	1.00E-151	100.00%	MT229085.1	mangrove plant
ACWB297-18	MA	AHW85L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-148	99.32%	MH201037.1	sponge

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB298-18	MA	AHW85L	Not reliable identification					
ACWB299-18	MA	AHW85L	a-proteobacteria	Pseudovibrio japonicus strain QS305	2.00E-153	98.72%	MK439596.1	Unknown
ACWB300-18	MA	AHW85L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-149	100.00%	MH201037.1	sponge
ACWB301-18	MA	AHW85L	a-proteobacteria	Pseudovibrio japonicus strain QS305	1.00E-151	99.67%	MK439596.1	Unknown
ACWB302-18	MA	AHW85L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	6.00E-154	99.67%	MH201037.1	sponge
ACWB303-18	MA	AHW86L	g-proteobacteria	Vibrio harveyi strain DS1907-aSP_2_1	2.00E-154	99.67%	MT269639.1	sea water
ACWB304-18	MA	AHW86L	g-proteobacteria	Psychrobacter marincola strain QS123	6.00E-144	98.32%	MK439597.1	Unknown
ACWB305-18	MA	AHW86L	g-proteobacteria	Psychrobacter marincola strain QS123	6.00E-144	98.32%	MK439597.1	Unknown
ACWB306-18	MA	AHW86L	Not reliable identification					
ACWB307-18	MA	AHW86L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB308-18	MA	AHW86L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB309-18	MA	AHW87L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB310-18	MA	AHW87L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB311-18	MA	AHW87L	a-proteobacteria	Pseudovibrio denitrificans strain Ps18	3.00E-157	98.75%	MH201038.1	sponge
ACWB312-18	MA	AHW87L	Not reliable identification					
ACWB313-18	MA	AHW87L	Not reliable identification					
ACWB314-18	MA	AHW88L	a-proteobacteria	Pseudovibrio sp. FO-BEG1	6.00E-159	99.06%	KX418559.1	marine sponge

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB315-18	MA	AHW88L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB316-18	MA	AHW88L	Not reliable identification					
ACWB317-18	MA	AHW88L	Not reliable identification					
ACWB318-18	MA	AHW89L	Not reliable identification					
ACWB319-18	MA	AHW89L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	5.00E-155	99.35%	MT187959.1	scleractinian coral
ACWB320-18	MA	AHW89L	Not reliable identification					
ACWB321-18	MA	AHW89L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	5.00E-155	99.35%	MT187959.1	scleractinian coral
ACWB322-18	MA	AHW89L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB323-18	MA	AHW810L	Not reliable identification					
ACWB324-18	MA	AHW810L	Actinobacteria	Dietzia maris strain I7	6.00E-159	98.76%	MT266927.1	Dietzia maris
ACWB325-18	MA	AHW810L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB326-18	MA	AHW810L	Not reliable identification					
ACWB327-18	10% MA	AHW81L	a-proteobacteria	Erythrobacter flavus strain 21-3	4.00E-151	99.67%	MN744319.2	sediment of cold seep
ACWB328-18	10% MA	AHW81L	Not reliable identification					
ACWB329-18	10% MA	AHW81L	Not reliable identification					
ACWB330-18	10% MA	AHW81L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB331-18	10% MA	AHW81L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB332-18	10% MA	AHW82L	Not reliable identification					
ACWB333-18	10% MA	AHW82L	g-proteobacteria	Pseudoalteromonas sp. ch212	8.00E-158	98.45%	LC379538.1	bottom sediments
ACWB334-18	10% MA	AHW82L	Not reliable identification					
ACWB335-18	10% MA	AHW83L	Not reliable identification					
ACWB336-18	10% MA	AHW83L	Not reliable identification					
ACWB337-18	10% MA	AHW83L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	3.00E-152	97.81%	MH201037.1	sponge
ACWB338-18	10% MA	AHW83L	Not reliable identification					
ACWB339-18	10% MA	AHW84L	Not reliable identification					
ACWB340-18	10% MA	AHW84L	Not reliable identification					
ACWB341-18	10% MA	AHW84L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-156	99.36%	MH201037.1	sponge
ACWB342-18	10% MA	AHW84L	a-proteobacteria	Pseudovibrio sp. strain 1701B84a	1.00E-156	99.36%	MG833243.1	Jaspis sponges
ACWB343-18	10% MA	AHW84L	g-proteobacteria	Pseudoalteromonas sp. strain MBL0373	5.00E-160	99.07%	MT187959.1	scleractinian coral
ACWB344-18	10% MA	AHW85L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	2.00E-154	99.04%	MH201037.1	sponge
ACWB345-18	10% MA	AHW85L	Not reliable identification					
ACWB346-18	10% MA	AHW85L	Not reliable identification					
ACWB347-18	10% MA	AHW85L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB348-18	10% MA	AHW86L	Not reliable identification					
ACWB349-18	10% MA	AHW86L	Not reliable identification					
ACWB350-18	10% MA	AHW86L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-155	99.67%	MH201037.1	sponge
ACWB351-18	10% MA	AHW86L	Not reliable identification					
ACWB352-18	10% MA	AHW86L	Not reliable identification					
ACWB353-18	10% MA	AHW86L	Not reliable identification					
ACWB354-18	10% MA	AHW87L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	3.00E-152	99.03%	MH201037.1	sponge
ACWB355-18	10% MA	AHW87L	g-proteobacteria	Pseudoalteromonas sp. T2	4.00E-156	99.04%	KR338872.1	Dicathais orbita (snail)
ACWB356-18	10% MA	AHW87L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	5.00E-155	99.04%	MH201037.1	sponge
ACWB357-18	10% MA	AHW87L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB358-18	10% MA	AHW88L	a-proteobacteria	Pseudovibrio sp. FO-BEG1	3.00E-152	98.41%	KX418553.1	marine sponge
ACWB359-18	10% MA	AHW88L	Not reliable identification					
ACWB360-18	10% MA	AHW88L	Not reliable identification					
ACWB361-18	10% MA	AHW88L	Not reliable identification					
ACWB362-18	10% MA	AHW88L	Not reliable identification					
ACWB363-18	10% MA	AHW88L	Not reliable identification					

Sample Name	Media	QIIME ID	Class	Description of Homologous Seq	E value	% ID	Accession #	Source
ACWB364-18	10% MA	AHW89L	Not reliable identification					
ACWB365-18	10% MA	AHW89L	Not reliable identification					
ACWB366-18	10% MA	AHW89L	Not reliable identification					
ACWB367-18	10% MA	AHW89L	Not reliable identification					
ACWB368-18	10% MA	AHW810L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB369-18	10% MA	AHW810L	a-proteobacteria	Erythrobacter atlanticus	3.00E-157	98.75%	LR722783.1	Coastal surface water
ACWB370-18	10% MA	AHW810L	Not reliable identification					
ACWB371-18	10% MA	AHW810L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB372-18	10% MA	AHW810L	g-proteobacteria	Psychrobacter pacificensis strain LPB0279	2.00E-154	99.67%	MN577288.1	Unknown
ACWB373-18	10% MA	AHW810L	a-proteobacteria	Pseudovibrio denitrificans strain Ps17	1.00E-156	98.75%	MH201037.1	Sponge

APPENDIX D:

GRAM STAIN

Bacteria that did not have reliable identification or could not be DNA sequenced were Gram-stained to determine if the culture was pure. A pure culture had only one shape of bacteria and was either Gram-positive or Gram-negative. If the sample had both Gram-positive and Gram-negative bacteria or more than one shape it was not a pure culture. If shape or Gram status was undetermined the purity of the culture was not determined.

Sample Name	Gram + or -	Shape of Bacteria	Pure culture?
XMEB003-18	Gram -	Coccus	Yes
XMEB005-18	Gram +	Coccus	Yes
XMEB006-18	Gram -	ND	ND
XMEB007-18	More than one type of Bacteria on agar		No
XMEB010-18	ND	ND	ND
XMEB012-18	Bacteria could not be tested		ND
XMEB014-18	Gram -	Coccus	Yes
XMEB018-18	More than one type of Bacteria on agar		No
XMEB019-18	Bacteria could not be tested		ND
XMEB020-18	Gram +	Coccus	Yes
XMEB021-18	Gram +	Coccus	Yes
XMEB024-18	More than one type of Bacteria on agar		No
XMEB025-18	More than one type of Bacteria on agar		No
XMEB026-18	Bacteria could not be tested		ND
XMEB027-18	More than one type of Bacteria on agar		No
XMEB028-18	Gram -	Coccus	Yes

Sample Name	Gram + or -	Shape of Bacteria	Pure culture?
XMEB030-18	Gram +	Coccus	Yes
XMEB031-18	Gram - and Gram +	Coccus	No
XMEB033-18	ND		ND
XMEB035-18	ND		ND
XMEB036-18	Gram -	Bacillus	Yes
XMEB037-18	Could not be determined	Coccus	Yes
XMEB038-18	Gram - and Gram +	Bacillus	No
XMEB039-18	Gram +	Bacillus	Yes
XMEB040-18	Bacteria could not be tested		Not determined
XMEB041-18	Gram - and Gram +	Could not be determined	No
XMEB045-18	Gram - and Gram +	Bacillus	No
XMEB052-18	Gram +	Bacillus	Yes
XMEB055-18	Gram -	Coccus	Yes
XMEB056-18	Gram - and Gram +	Could not be determined	No
XMEB057-18	Gram +	Bacillus and coccus	No
XMEB059-18	Bacteria could not be tested		Not determined
XMEB062-18	Gram - and Gram +	coccus	No
XMEB063-18	Gram - and Gram +	Could not be determined	No
XMEB064-18	Gram - and Gram +	Could not be determined	No
XMEB065-18	Gram - and Gram +	Could not be determined	No

Sample Name	Gram + or -	Shape of Bacteria	Pure culture?
XMEB066-18	Gram - and Gram +	Could not be determined	No
XMEB067-18	Gram -	Could not be determined	Not determined
XMEB070-18	Gram - and Gram +	coccus	No
XMEB071-18	Gram -	bacillus	Yes
XMEB073-18	Gram -	Could not be determined	Not determined
XMEB075-18	Gram - and Gram +	coccus	No
XMEB076-18	Gram -	bacillus	Yes
XMEB078-18	Gram -	coccus	Yes
XMEB079-18	Gram - and Gram +	coccus	No
XMEB080-18	Gram +	Bacillus	Yes
XMEB081	Bacteria could not be tested		Not determined
ACEB086-18	Gram +	Bacillus	Yes
ACEB087-18	Gram +	Bacillus	Yes
ACEB091-18	Gram -	Could not be determined	Not determined
ACEB095-18	Could not be determined	Coccus	Not determined
ACEB096-18	Gram - and Gram +	Could not be determined	No
ACEB097-18	Gram +	Coccus	Yes
ACEB099-18	Could not be determined	Coccus	Not determined
ACEB100-18	Gram -	Could not be determined	Not determined
ACEB102-18	Gram - and Gram +	Could not be determined	No
ACEB103-18	Gram +	Bacillus	Yes

Sample Name	Gram + or -	Shape of Bacteria	Pure culture?
ACEB104-18	Gram +	Bacillus	Yes
ACEB105-18	Gram +	Could not be determined	Not determined
ACEB106-18	Gram -	Coccus	Yes
ACEB108-18	Gram - and Gram +	Coccus	No
ACEB112-18	Gram -	Bacillus and Coccus	No
ACEB131-18	Gram -	Could not be determined	Not determined
ACEB134-18	Gram -	Could not be determined	Not determined
ACEB135-18	Gram -	Could not be determined	Not determined
ACEB142-18	Could not be determined	Could not be determined	Not determined
ACEB155-18	Bacteria could not be tested		Not determined
ACEB161-18	Gram - and Gram +	Coccus and bacillus	No
ACEB169-18	Could not be determined	Coccus	Not determined
ACEB172-18	Gram +	bacillus	Yes
ACEB179-18	Could not be determined	Coccus	Not determined
XMWB195-18	Gram - and Gram +	Coccus	No
XMWB201-18	More than one type of Bacteria on agar		No
XMWB203-18	More than one type of Bacteria on agar		No
XMWB225-18	Gram - and Gram +	Could not be determined	No
XMWB228-18	Gram - and Gram +	Could not be determined	No
XMWB231-18	Could not be determined	Coccus	Not determined

Sample Name	Gram + or -	Shape of Bacteria	Pure culture?
XMWB233-18	Gram - and Gram +	Coccus and Bacillus	No
XMWB235-18	Bacteria could not be tested		Not determined
XMWB239-18	Gram - and Gram +	Could not be determined	No
XMWB240-18	Gram -	Could not be determined	Not determined
XMWB241-18	Gram - and Gram +	Coccus	No
XMWB243-18	Gram - and Gram +	Coccus and Bacillus	No
XMWB255-18	Gram -	Could not be determined	Not determined
XMWB257-18	Gram - and Gram +	Could not be determined	No
XMWB258-18	Gram +	Could not be determined	Not determined
XMWB259-18	Gram -	Bacillus	Yes
ACWB262-18	Could not be determined	Coccus	Not determined
ACWB264-18	Gram - and Gram +	Could not be determined	No
ACWB265-18	Gram -	Bacillus	Yes
ACWB266-18	Gram - and Gram +	Bacillus	No
ACWB271-18	Gram - and Gram +	Coccus	No
ACWB275-18	Gram -	Could not be determined	Not determined
ACWB284-18	Bacteria could not be tested		Not determined
ACWB290-18	Gram -	Bacillus	Yes
ACWB306-18	Gram -	Coccus	Yes
ACWB313-18	Gram -	Coccus	Yes

Sample Name	Gram + or -	Shape of Bacteria	Pure culture?
ACWB326-18	Gram - and Gram +	Coccus and Bacillus	No
ACWB332-18	Gram - and Gram +	Coccus and Coccus, Bacillus	No
ACWB334-18	Gram +	Could not be determined	Not determined
ACWB335-18	Gram +	Coccus	Yes
ACWB338 -18	Gram +	Bacillus	Yes
ACWB340-18	Gram +	Coccus	Yes
ACWB346-18	Gram - and Gram +	Could not be determined	No
ACWB351-18	Gram -	Coccus and Bacillus	No
ACWB353-18	Could not be determined	Could not be determined	Not determined
ACWB359-18	Could not be determined	Could not be determined	Not determined
ACWB367-18	Gram - and Gram +	Could not be determined	No
ACWB370-18	Gram +	Coccus	Yes