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Microbial Properties of *Montipora* Coral Mucus

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ABSTRACT

Mucus produced by corals has a varied understanding with many gaps. Although there are many hypotheses regarding the function and purpose of the mucus itself, and the many microbial communities that inhabit it, there are still many inquiries about it. The purpose of this experiment was to determine whether coral mucus possessed diverse microbes and whether the mucus held any antibacterial properties against gram positive or gram negative bacteria compared to seawater. Mucus was collected from *Montipora* species and plated on marine agar. Individual bacterial colonies were isolated and gram stained. Our results showed that the mucus does not show antibacterial activity but contains as much as five times the amount of bacteria in comparison to seawater. Additionally, the coral mucus contained exclusively gram negative bacteria whereas the seawater contained both gram negative and gram positive bacteria. These results suggest that coral mucus is a hospitable site for growth of gram negative bacteria.

1 INTRODUCTION

Mucin, a macromolecule consisting of various glycoproteins, plays an active role in many biological processes. While the exact purpose varies greatly among these processes, its presence is often indicative of a specific function. Mucus plays an active role in response to diseases by allowing for specific pathogens to bind to it, and thus preventing the pathogens from penetrating the target organism. In pharmacology, it is understood that mucus acts as a barrier, which nutrients and drugs must interact with and diffuse through in order to be effective (Bansil & Turner, 2006).

The marine environment contains a unique, distinct microbe composition. In the marine environment, the microbial organisms that inhabit mucus are of particular interest. This allows certain microbes to participate in potentially symbiotic relationships in the biological processes of marine organisms responsible for synthesizing the mucus. For example, mucus has been commonly observed on fish as a protective layer against pathogens while also promoting the growth of certain bacteria. The mucus that coats fish is highly selective and is unique to each species. The mucus is capable of discriminating between pathogenic bacteria as well as commensal bacteria (Benhamed et al., 2014). Looking beyond pelagic marine organisms mucus as a desirable microbial environment, our attention was drawn to other microbe populations that reside within benthic regions. Depending on the marine niche, microbe content varies significantly. Specifically within coral associated niches, there is a higher abundance of bacteria present. Within the water of coral reefs, there is a high microbial abundance and activity, but it varies slightly from each part of the reef (Tout et al., 2014). Stony corals also produce a great volume of mucus that hosts a diverse microbial population, potentially analogous to that of fish.

The coral holobiont is a complex community consisting of zooxanthellae and many microbial communities (Golberg et al., 2011). While this mucus potentially holds a great value, there is a lack of complete understanding of the composition of coral mucus, namely its structure and synthesis. Furthermore, identifying the exact purpose that underlies its synthesis is still unclear. However, there are many studies that have investigated its potential role. The mucus has many beneficial functions for corals, which include feeding, protection from pathogens, sediment removal and potentially others that have yet to be identified (Ducklow & Mitchell, 1979). The mucus has shown to have antibacterial properties against certain bacteria, while enhancing the growth of others (Shnit-Orland et al., 2012). Also, there are theories regarding the possibility of quorum sensing and a correlation to the formation of a biofilm, which would provide protection to invading pathogens (Tout et al., 2014). Ultimately, it is difficult to identify one specific function of coral mucus. In this experiment we sought to investigate whether the mucus possessed diverse microbes and whether possible antibacterial properties were present within the mucus compared to seawater.

2 METHODS AND MATERIALS

Bacterial Cultures from Mucus

A sample of mucus from fragmented Montipora was obtained from a local aquarium, as well as a sample of marine water from the same tank. Two marine agar (MA) plates were inoculated using a sterile loop; one MA plate was inoculated with the mucus (Plate A) and one was inoculated with the marine water (Plate B). Both plates were then incubated at 37 °C in a New Brunswick Scientific Co. incubator for 48 hours. Following an incubation period of 48 hours, Plates A and B were obtained from the incubator and growth was observed on both plates. The streak plate technique was then used as a method for isolating the bacterial colonies observed on both plates.

Ten-fold serial dilutions were performed to quantify the amount of bacteria present in the coral mucus compared to the seawater. Four microcentrifuge tubes were obtained and labeled I–VI. 900 µL of sterile water was transferred into each tube using a pipet. Using a sterile loop, tube I was inoculated from mucus collected from the same specimen which was plated on plate A. Then, 100 µL of solution was transferred from I to II using a pipet. This step was repeated for tubes III–VI. Six MA plates were obtained and labeled I–VI. 25 µLof solution was transferred from each tube to the MA plate of corresponding value. Using a sterile cell spreader, the solution was spread over the MA plates. Each MA plate (I–VI) was then incubated at 37 °C in a New Brunswick Scientific Co. incubator for 24–48 hours and then placed in a refrigerator until viewing. The same process was repeated, for the sample used on plate B to quantify the amount of bacteria in sea water.

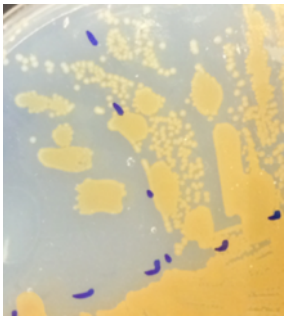
Isolate	Gram Positive/Negative	Shape	Description	Picture
SP-A	Negative	Bacillus	Orange pigmentation, whole, raised, circular	
SP-B	Positive	Bacillus	White pigmentation, circular, whole, entire, raised	

Table 1. Gram Stain of Isolate SP-A and SP-B

Two new MA plates were obtained, streaked, and labeled Streak Plate A (SP-A) and Streak Plate B (SP-B) for the mucus and salt water, respectively. A colony from plates A and B were chosen to be streaked to observe individual morphology. By carefully lifting one edge of the Petri plate cover, the first sector of the plate was streaked without overlapping previous streaks. Next, the loop was flamed and allowed to cool. The loop was used to carefully streak through one area of the first sector, and then into the second sector. This process was completed for a total of four sectors, avoiding contact with streaks from previous sections while streaking the final sector. SP-A and SP-B were then incubated at 37 °C in a New Brunswick Scientific Co. incubator for 24–48 hours and then placed in a refrigerator until viewing. SP-A and SP-B were obtained from the incubator 24 hours after streaking, and isolated colonies were observed on both plates. In order to identify and classify the isolated colonies, a Gram stain was performed for SP-A and SP-B. To determine morphology, the stains were analyzed using a compound light microscope at 1000× magnification.

Antibiotic Production

The disk-diffusion method was performed to screen for any antimicrobial properties in the mucus. Two nutrient agar (NA) plates were inoculated using a sterile swab; one with *Escherichia coli* and the other with *Staphylococcus aureus*. The swab was passed many times over the plate to create a lawn of bacteria. The NA plates were divided into four quadrants. In quadrant I, a blank paper disk was placed onto the agar. A disk impregnated with mucus, a disk impregnated with seawater, and a disk impregnated with quaternary detergent (lab disinfectant), were placed in quadrants II, III, and IV, respectively. Both NA plates were then incubated at 37 °C in a New Brunswick Scientific Co. incubator for 24–48 hours and then placed in a refrigerator until viewing.

Species	Impregnated Paper Disk	Zone of Inhibition (mm)
Staphylococcus aureus	Control (I)	0
	Mucus (II)	0**
	Seawater (III)	0
	Quat. Detergent (IV)	8
Escherichia coli	Control (I)	0
	Mucus (II)	0**
	Seawater (III)	0
	Quat. Detergent (IV)	4

Table 2. Antibiotic Production Quantified by Disk Diffusion of SP-A and SP-B

3 RESULTS

Three unique colonies were observed on plate A (mucus). One of the colonies observed was white in pigmentation, irregularly formed, had flat elevation and an undulate margin. The other colony observed was also white in pigmentation, while it had a filamentous form, flat elevation and lobate margin. One particular colony, which appeared in great abundance and more frequently than the others,

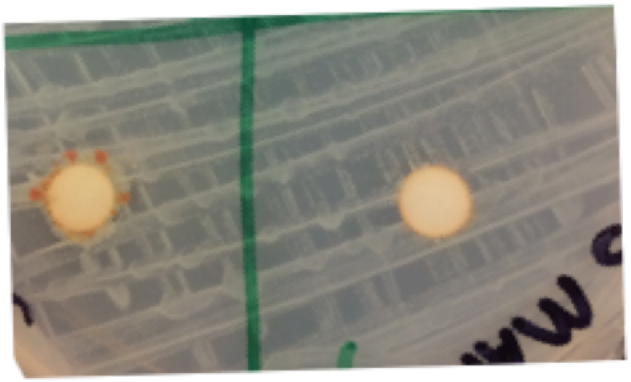


Fig. 1. Additional bacteria growth produced on disk diffusion.

Dilution	Plate Number	Solution	Number of Colonies (CFU/mL)
10^{-1}	I	Mucus Seawater	TNC TNC
10^{-2}	II	Mucus Seawater	TNC 16×10^4
10^{-3}	III	Mucus Seawater	19×10^5 33×10^3
10^{-4}	IV	Mucus Seawater	5×10^6 9×10^5
10^{-5}	V	Mucus Seawater	7×10^6 0
10^{-6}	VI	Mucus Seawater	0 0

Table 3. Antibiotic Production Quantified by Disk Diffusion of SP-A and SP-B

were the colonies recorded as orange with a circular whole-colony appearance, entire margins, and a raised elevation. We chose to investigate the properties of these colonies due to their populous presence. One type of isolated colony was also observed from plate B (seawater). The appearance of the colonies were described as being white with a circular whole-colony appearance, entire margins, and a raised elevation. The stains from A (mucus) and B (seawater) were analyzed using a compound light microscope at 1000× magnification. The morphology of the bacteria in SP-A was recorded as bacillus shaped, and Gram positive. The morphology of the bacteria in SP-B was recorded as bacillus shaped and Gram negative (Table 1).

The two NA plates used in the disk-diffusion method were obtained from the incubator. As shown in Table 2, Quaternary detergent (IV) was used as a positive control and was the only quadrant that displayed a zone of inhibition of 8 mm and 4 mm for *S. aureus* and *E. coli*, respectively (Table 2). The mucus displayed a different unexpected result. The mucus enhanced bacterial growth, and multiple orange colonies appeared in response to the *S. aureus* and *E. coli* (Figure 1). The disk adjacent, was saturated with saltwater, shows no additional growth.

The dilution plates were obtained and the number of colonies were recorded. The 10^{-1} dilutions for both solutions grew a tremendous number of colonies and were too many too count. 160 colonies were counted for the seawater solution for the 10^{-2} dilution, and there was too many to count for the mucus. At 10^{-3} dilution, 185 and 33 colonies were counted for mucus and seawater, respectively. At 10^{-4} dilution, 52 and 9 colonies were counted for mucus and seawater, respectively. The more diluted plates (10^{-5} and 10^{-6}) did not contain a countable number of colonies (25–250) (Table 3).

4 DISCUSSION

Our results demonstrated that there were more microbes present in the mucus than the seawater (Table 3). Additionally, we found many diverse bacteria inhabiting the mucus as well as seawater (Table 1). Upon performing a gram stain from isolated colonies, it was found that these colonies proved to be gram negative on the coral, while the surrounding water favored gram positive bacteria. In this experiment, the results obtained showed that the coral mucus does not possess any antibacterial properties due to a lack of a zone of inhibition (Table 2). However, this does not mean that the mucus does not possess any anti-microbial properties. Further research with alternative methods should be done in this area. Ultimately, it can be concluded that the mucus holds a great deal of potential symbiotic bacteria that live on the coral holobiont. However, we cannot determine from our study whether these bacterial species act as mutualists, commensals, or parasites within the coral mucus.

Although we did not observe antibacterial properties of *Montipora* mucus, previous research suggested that the mucus and its microbes are in fact antibacterial. According to the previous literature by Shnit-Orland et al. (2012), around five strains of *Pseudoalteromonas* were present in many different coral species' mucosa. *Pseudoalteromonas* displays antibacterial activity against gram positive, but not gram negative bacteria. It was found that it inhibited the growth of *Bacillus cereus* and *Staphylococcus aureus* (Shnit-Orland et al., 2012). Furthermore, what was observed on the mucus disks inoculated with *Staphylococcus aureus*, was the growth of an orange bacteria. An identifying trait of *Pseudoalteromonas* is an orange pigment. Even though we were not able to identify the bacteria as *Pseudoalteromonas*, the unknown bacteria's orange hue matches that of *Pseudoalteromonas* (Shnit-Orland et al., 2012). Although we did not observe inhibition of *Staphylococcus aureus* in our assay, the presence of this bacteria may indicate that *Pseudoalteromonas* may restrict growth of other microbes through microbial antagonism in the host.

After performing serial dilutions of the mucus and comparing it to the seawater, our results show that there was five-fold the amount of bacteria present in mucus than in the water column.

This finding is consistent with the literature produced on this topic. According to previous research done by Garren & Azam (2011), the amount of bacteria present in mucus is six times the amount of that of the surrounding seawater8. It is hypothesized that this is a result of the properties of the mucus. Mucus has been proven to be very nutrient rich, high in ammonium, phosphate, organic carbon and nitrogen (Bythell & Wild, 2011). Despite being nutrient rich, it is also selective for gram negative bacteria. The literature regarding this topic is currently limited, with little understanding about the interactions between bacterial communities on their host corals (Golberg et al., 2011).

We were able to isolate a several unique colonies from the mucus on the plate and identify their cell wall structure via gram stain. They show that the bacteria present on the mucus was mostly gram negative, while the water exhibited mostly gram positive bacteria. Despite these limited findings in our study, the results of other studies on this topic are vary. However, a higher prevalence of gram negative bacteria present on reefs has been documented (Taniguchi et al., 2014). This finding would be consistent with the results found by Shnit-Orland et al. (2012), regarding how the microbe *Pseudoalteromonas* has antibacterial effects against gram positive bacteria.

The data presented here have several limitations. First, the coral mucus sample size was very small. The *Montipora* used for collection was from the same tank and the same coral multiple times. This means our data produced insufficient results on diverse microbes content compared to data collected from corals that reside in the ocean (Bettarel et al., 2016). Secondly, we only focused on one species of coral, *Montipora*, when in actuality it would be interesting to look for microbial presence across a wide spectrum of species for variation and similarities. Since we were able to confirm the microbial activity present in the mucus, this study serves as additional evidence to support the coral probiotic hypothesis. This hypothesis states that the microbial community will change in order to allow for the coral holobiont to adapt to various stressors (Taniguchi et al., 2015). This hypothesis can be used in future research in regards to testing coral mucus microbe activity in corals cultured in captivity compared to the ocean. Currently, it is understood that coral health is on a large decline due to multiple environmental stressors. If a full understanding of coral probiotics was completed it might provide a solution to the declining health of corals.

REFERENCES

Bansil, R. & Turner, B.S. 2006, J Exp Mar Bio Ecol 11:164–170

Benhamed, S., Guardiola, F.A., Mars, M. & Esteban, M.A. 2014, Vet Microbiol 171(1):1–12

Bettarel, Y., Thanh, M.C., Patrice, G., Antoinette, A., Nadege, K.N., Bui, V.N. & Thierry, B. 2016, J Microbiol Meth 128:16–19

Bythell, J.C. & Wild, C. 2011, J Exp Mar Bio Ecol 408:88–93

Ducklow, H.W. & Mitchell, R. 1979, Limnol Oceanogr 24:715–725

Garren, M. & Azam, F. 2011, ISME J 6:1159–1165

Golberg, K., Eltzov, E., Shnit-Orland, M., Marks, R.S. & Kushmaro, A. 2011, Microbial Ecol 61(4):783–792

Shnit-Orland, M., Sivan, A. & Kushmaro, A. 2012, J Exp Mar Bio Ecol 64:851–856

Taniguchi, A., Yoshida, T. & Eguchi, M. 2014, J Exp Mar Bio Ecol 461:331–336

Taniguchi, A., Yoshida, T., Hibino, K. & Eguchi, M. 2014, J Exp Mar Bio Ecol 469:105–112

Tout, J., Jeffries, T.C., Webster, N.S., Stocker, R., Ralph, P.J. & Seymour, J.R. 2014, J Exp Mar Bio Ecol 67:540–552