Identification of bacterial species growing on aged *Nereocystis luetkeana* and *Agarum fimbriatum.* in the San Juan Channel

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Abstract: Do bacterial communities change as algae decays? If so, how does it change? Bacteria form many different types of relationships with algae and this study observed the effects of algal decay on bacterial abundance and composition on *Nereocystis luetkeana* and *Agarum fimbriatum*. I extracted and cultivated bacteria from algae collected in the San Juan Channel. I then counted colony numbers and monitored colony types based on morphological features. These observations are important for understanding feeding preferences of fresh vs. aged algae in nearshore organisms. If we can start to understand the relationship between decaying algae and the bacterial communities present on them, we can also start to understand the reason for food preference.

Introduction:

Bacteria are one of the earliest life forms to appear on Earth and have been found in most habitats on the planet; including soil, water, and in the bodies of plants and animals. Consequently, bacteria are able to form mutualistic relationships with their hosts. For example; *Bacteroides thetaiotamicron* inhabits the human intestinal tract and breaks down otherwise indigestible polysaccharides (Backhed et al., 2005). Actinomycetes bacteria have been observed to form nitrogen-fixing endosymbiotic relationships with legumes (Richardson et al., 2000). However, in the marine environment, little is known about the relationship between bacteria and algae.

There has been documentation of the possible role of bacteria in enhancing the growth of algae (Cid et al. 1992, del Giorgio et al. 1997, Moreno & Laine 2004, de

Kluijver et al. 2010). Arora et al. (2012) found that the inoculation of *Tetraselmis indica* with bacteria "had a pronounced effect on algal growth, which either decreased (as observed with *Pseudomonas sp.*) or increased (as observed with *Acinetobacter sp.*)". In the same study, prolonged survival of *T. indica* was observed following inoculation with *Rugeria* sp. and *Acinetobacter* sp. It has also been proposed that in periods of carbon limitation, bacteria may be a significant source of CO2 for algal growth (Lange, 1970, 1971). Hence, there is a complex relationship between algae and bacteria, that can either a benefit or detriment to the algae.

My research aims to answer three primary questions: Do bacterial communities change as algae decay? 2) If so, how does bacterial composition change and/or do population numbers differ?, and 3) Are the bacteria growing on decaying algae the same as the bacteria found in surrounding waters?

Methods:

The algae used for this study, *Nereocystis luetkeana* and *Agarum fimbriatum*, were collected in the waters of the Salish Sea. As the algae degraded, weekly samples of bacteria from the surface of algae were collected and cultivated on agar plates. Abundance and biodiversity counts were monitored in order to determine changes in population numbers and population composition. After collection was complete, bacterial strains were isolated, DNA extracted from the isolated colonies, and sequenced. Finally, the sequences were compared to the GenBank database for identification.

Seawater was also collected near the algal collection sites for a qualitative comparison of the bacteria found on the algae and in the water. Algal aging took

place in flow-through sea-tables, with continuous flows of seawater and kept under dark conditions. Each type of algae had separate aging tanks to avoid any cross contamination of bacteria. The algae were kept inside mesh bags, which were then placed in buckets with holes, to avoid the acceleration the physical breakdown of my samples by the water current. The algae aged for three weeks. Each week I sampled three pieces of algae from each alga. To extract the bacteria from the algae, 5x5 mm pieces of algae were blotted and air-dried. The pieces were placed in 10 mL of sterile seawater and shaken in a vortex mixer to dislodge the bacteria from the surface of the algae. Water from each of the holding tanks was also sampled weekly as controls.

I diluted the supernatant of the algae/seawater mix by factors of 10, 100, 1000, and 10,000. 100 µl of each dilution were streaked on agar plates. Agar was poured in lab with a mixture of 2216 medium marine broth (Difco) and BD Bacto™ Agar. After 5-8 days of incubation at 23°C, colony population counts and morphologically different colonies were recorded. By taking the population count and multiplying it by the dilution factor, I obtained the "Most Probable Number" of cultivatable bacteria present in the sample (an estimate of the number of bacteria from the 5x5 mm piece of algae). Based on differences in colony size, shape, and color; the colonies were assigned to Operational Taxonomic Units (OTUs).

I then isolated and cultivated the most numerous types of bacteria from each time point, transferring colonies onto new plates and incubating them at 23° C. After significant growth occurred, I extracted DNA from the colonies using a Mo Bio Labs - UltraClean™ Microbial DNA isolation kit and amplified the 16s ribosomal RNA gene

via PCR. Subsequently, PCR products were sequenced by GeneWiz. Finally, I ran a BLAST search on the sequences and obtained the GenBank accession numbers for identification.

Results:

Population Numbers

Bacterial population numbers in *Agarum* stayed relatively constant throughout the experiment (Fig.1). I observed an average population of 0.1 x 10^6 in the initial collection trial, 0.04 x 10^6 in week 1, 0.01 x 10^6 in week 2, and 0.23 x 10^6 in week 3 (Fig.2). *Nereocystis* microbial population numbers increased dramatically after two weeks of aging. I observed an average population of 0.04 x 10^6 in the initial collection trial, 0.03 x 10^6 in week 1, and 2.23 x 10^6 in week 2 (Fig.3). There is no data for the week 3 trial of *Nereocystis*. *Nereocystis* showed little growth at the initial collection and week 1 trials. There is no week 3 data for *Nereocystis* due to the rapid degradation of my sample.

Population Composition

The bacterial composition on *Agarum* did not vary much from week to week, in each trial there are two types of bacteria (Fig. 2). There was an anomaly in week 2 of the *Agarum* trial that showed no growth on any of the plates. *Nereocystis* microbial composition showed a large increase in week two of the experiment with a shift in the dominant bacterial species (Fig.3).

Discussion:

Comparison of bacteria found on my samples vs. the water controls:

The third of my hypotheses was set up to determine whether or not bacteria abundant in my treatments were also abundant in the surrounding water. I hypothesized that the bacteria found on the algae were different than bacteria found in the water. Through my observations I accepted the null hypothesis of no difference between the bacteria on the algae and the bacteria in the surrounding water. In my experiment, I observed that three of the five types of bacteria found on *Agarum* were also found in the control treatments (Table 1). The data for *Agarum* also shows that bacteria found in the controls is found at all stages of degradation. For *Nereocystis*, only one of the ten types of bacteria found on the algae was also observed in the control treatments (Table 2). The one bacterial type found in both the control and treatment could imply secondary colonization. Bacteria are among the early colonizers of macroalgae and can produce cues that stimulate or deter subsequent settlement of detrimental secondary colonizers (Lachnit et al. 2011, Egan et al. 2013).

My results show that *Agarum* is a poor host for bacteria, as no trend in population numbers was observed (Fig.1) and population composition stayed constant (Fig.2). I also hypothesized a change in bacterial abundance and population composition as algae ages. For *Agarum,* I accepted the null hypotheses of no change in abundance and composition. This supports previous work, in which microbial densities in *Agarum* did not show any trends with time and maintained low microbial counts (Sosik, 2011). *Agarum* produces phlorotannins, which are believed

to act as a deterrent to microbial colonization and herbivory (Iken et al. 2009). Sosik (2011) also suggests that phlorotannins are not continually produced in detrital blades, but that the phlorotannin compounds are resistant to breakdown. This idea is supported by Goecke et al. (2010), in which macroalgae prevent damage from symbiotic bacteria by producing secondary metabolites.

Agarum was also observed to have decayed slower than *Nereocystis* and it has been hypothesized that secondary metabolite defenses of macroalgae might reduce epibiosis and inhibit premature decomposition (Engel et al, 2006). The composition of the bacterial populations on macroalgae is influenced to some extent by the surface chemistry of the alga, as algal metabolites and surface tissue can selectively attract or repel bacteria (Lachnit et al. 2011). These observations might be understood better with further research into the interactions of phlorotannin and microbial populations, focusing on the bacteria that I found in the waters of the San Juan Channel.

In the initial and week 1 *Nereocystis* samples, there was very little growth on any of the plates. In the 2nd week of aging there was a population boom of both abundance and composition. This observation led me to reject the null hypotheses of no change in abundance and composition. This may have been a result of my experimental methods. When handling the *Nereocystis*, I observed a layer of slime coating the surface of the algae. My methods involve blotting the algae dry before starting my trials to rid the surface of any seawater, and consequently the layer of slime with it. The bacterial growth I found in week 2 could be a result of my sample piece of *Nereocystis* halting the production of the slime layer. Further studies could

focus on the mechanisms that produce that layer of slime, in order to find out when *Nereocystis* ceases to expel that layer. It would also be beneficial to study the interaction between bacteria and the slime coating.

The observations made of *Nereocystis* have many implications on the feeding preferences of nearshore organisms. A preference for aged algae could imply a selection for certain types of bacteria. Additionally, a preference for aged algae could also imply nutritional value added by the increase in bacterial population numbers. For future studies, the cataloguing of bacterial types is crucial. With an understanding of the microbial changes occurring on aging algae and identifications of the bacteria present, we can start to deduce the components of feeding preference.

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Figures:

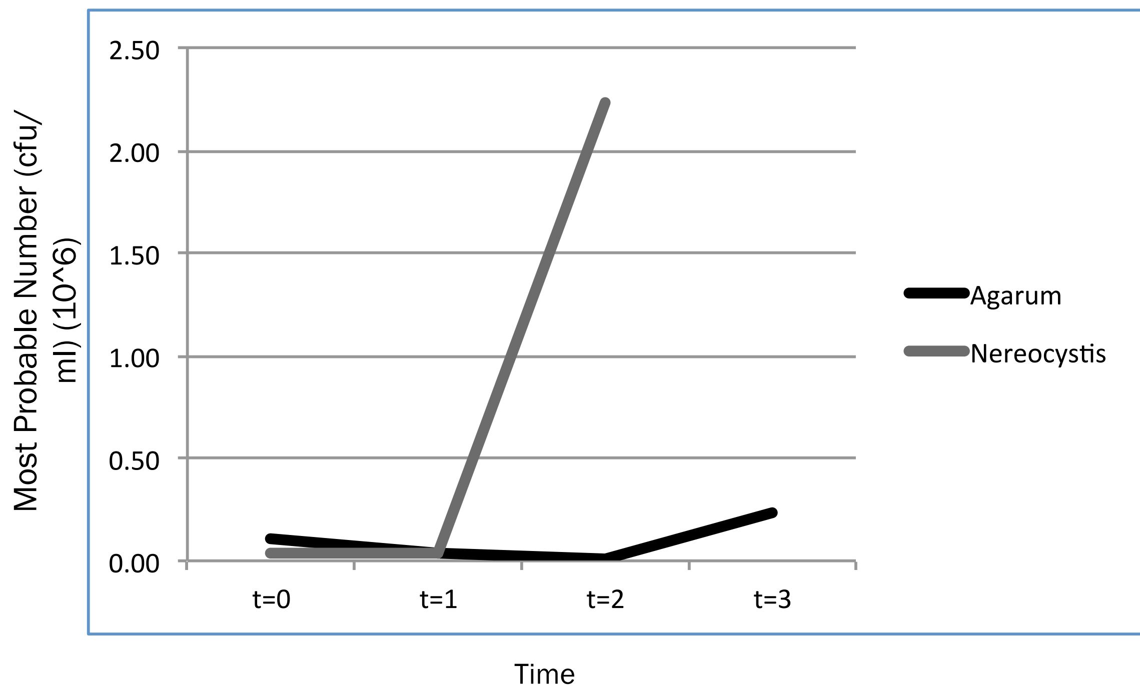

Figure 1: Mean microbial populations trends over time. Most Probable Number is an estimate of the number of bacteria present on the sample piece of algae.

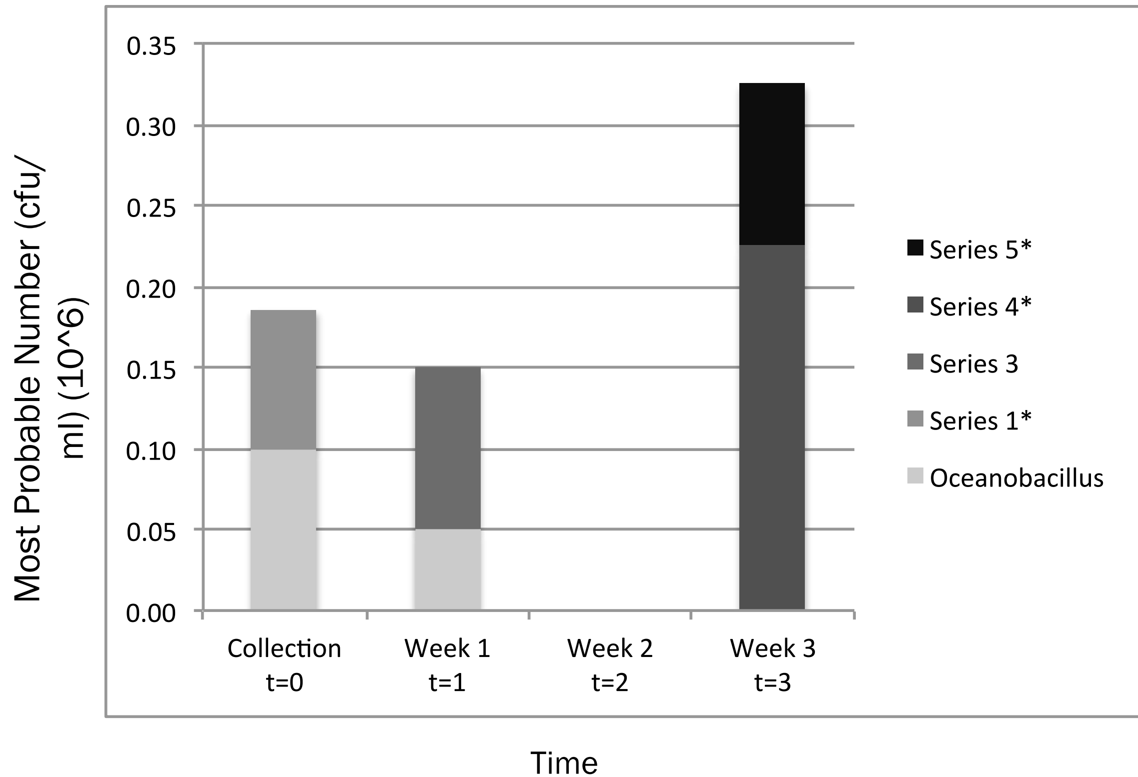

Figure 2: ***Agarum*** **bacterial composition. Unidentified bacterial types are labeled as "series". Most Probable Number is an estimate of the number of bacteria present on the sample piece of algae. (*) indicates the bacterial type was found in the controls.**

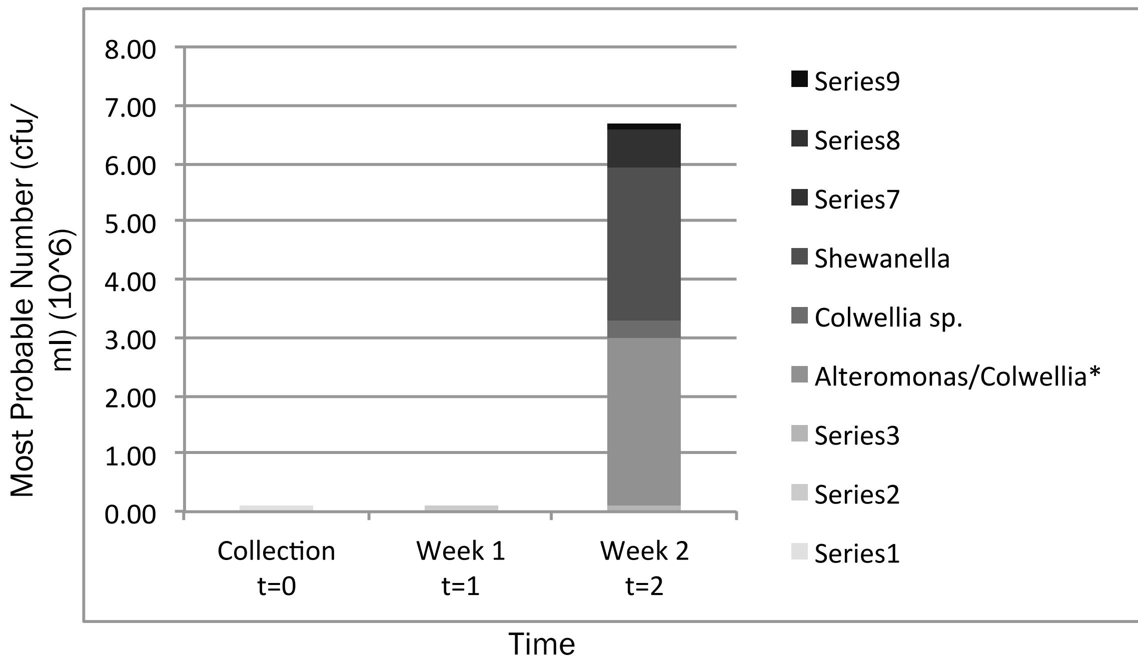

Figure 3: ***Nereocystis luetkeana*** **bacterial composition. Unidentified bacterial types are labeled as "series". Most Probable Number is an estimate of the number of bacteria present on the sample piece of algae. (*) indicates that the bacterial type was found in the controls.**