Micro-Cities: Bacterial Biofilms

By Kristina Mojica, UH Sea Grant-supported graduate student

Bacteria are ubiquitous; existing everywhere, all the time. What you might not know is that in aqueous environments bacteria tend to settle onto surfaces and form micro-cities called biofilms. Much like in our own cities, there are concrete structures which protect the bacteria they house from harsh currents, temperature, antibiotics, biocides, and even immune systems. The concrete from which these plume or streamer structures are constructed is exopolysaccharides, or complex sugars. Water channels mimic streets, transporting water and nutrients into the biofilm and carry out wastes. So how do such seemingly simple creatures form such complex communities? They talk of course!

Bacterial communication is termed quorum sensing. Unlike most creatures, bacteria do not use sound, but converse via molecular compounds. Biofilm formation is "discussed" through the use of acylated homoserine lactone (AHL) molecules. The process begins once bacteria settle onto a surface, as the number of bacteria increase, so does the concentration of these freely diffusible molecules. In this manner, the bacteria can "hear" the presence of each other. Once the bacteria distinguish that they have reached a population big enough to build their city without their efforts being wasted, they begin to build. The process is actually initiated when the AHLs bind to receptor molecules (i.e., the ears of the whole system); this new complex can then activate target genes that encode for biofilm phenotype (the appearance or behavior manifested by the activated gene). The bacteria, now distinctly different than their planktonic form (free swimming), produce large quantities of exopolysaccharides which enclose the bacteria in a protective matrix.

Biofilms are found almost anywhere there is water. From the slime on your teeth, where they cause plaque and contribute to the formation of cavities, to the slime on the rocks of streams, to the soil on the ground; biofilms are found everywhere. One of the most serious concerns about biofilms is that they can harbor and protect pathogenic (disease-causing) bacteria making them resistant to the treatment of antibiotics, biocides and your immune system. As a result of this protection, biofilms play an implemental role in water contamination, implant infections, ear infections and the disease cystic fibrosis. If our normal weapons are powerless, how are we to fight against these bacterial barricades?

Growth curves for biofilm formations are obtained by measuring biofilms formed in test tubes.

In the ocean, where there are more than a million bacteria per tablespoon of seawater, biofilms are unavoidable. Any surface submerged in the ocean for an extended amount of time will develop a biofilm. However, this is only part of the problem. The biofilm acts as a conditioning agent for surfaces, making them more desirable for other organism to settle on. In this manner, biofilms can lead to the process of biofouling. Biofouling is both necessary for marine ecology and problematic for human objects placed in the marine environment.

Biofouling can also be a problem for marine plants. The presence of biofilms and biofouling can block light from reaching the photosynthetic surfaces of plants and can negatively affect growth. But at least one plant, *Delisea pulchra*, has devised an ingenious way of preventing this dilemma. This Australian red algae produces its own molecular compounds, halogenated furanones, that mimic the AHL of the bacteria. It displays these molecules on its photosynthetic surfaces, where they act as ear plugs to bacteria which settle here, blocking the receptor molecules from binding to AHL molecules and activating the biofilm genes. Thus, biofilm formation is not initiated and photosynthetic surfaces remain clean. The existence of the halogenated furanones is exciting because it opens the possibility for controlling biofilm formation. An ability that can lead to the development of new drugs to treat biofilm based diseases or engineering of new non-toxic technologies to prevent biofouling. So, the battle against biofilms is far from over.

This is where my research comes in. I have been developing an assay that can measure biofilm growth as a function of the production of exopolysaccharides. The assay was developed using a bacteria isolated from a natural biofilm from waters off O'ahu. The bacteria are used to form single species biofilms over set allotments of time. I then measure the amount of exopolysaccharides produced using a chemical reaction that produces a chromagen that can then be measured spectrophometrically. To date, I have been able to reproducibly tract biofilm growth over a period of 72 hours. The overall goal of my research is to provide a simple and reliable way to screen marine plants for the ability to control biofilm formation.

Once a reliable screening technique is established, it can then be implemented to discover new plants and compounds that have this amazing ability to breakdown the biofilm barrier. The waters of Hawai'i offer a rich and promising source of marine plants. Hawai'i has been geographically isolated for millions of years; as a result, it is home to many endemic species. In fact, as much as 25 percent of Hawai'i's marine flora is endemic ranging from common to extremely rare. This, combined with the lack of scrutiny that has been applied to Hawai'i marine flora, makes the hope of discovering plants with novel abilities very promising. This could only mean good things for both Hawai'i and the battle against bacterial cities.

Kristina Mojica and mentor Dr. Michael J. Cooney, UH Sea Grant research faculty.

As a member of the University of Hawai'i Sea Grant College Program's (UH Sea Grant's) network of researchers and scientists, all supported graduate students are required to share their work with the local community. This outreach component of a student's time with UH Sea Grant is key in disseminating pertinent scientific information regarding coastal and marine resources to the public in a meaningful way.

In partnership with her advisor, Dr. Michael Cooney, Kristina Mojica has not only fulfilled this UH Sea Grant requirement, but has taken it a step further in creating a comprehensive laboratory curriculum for Hawai'i state high school students and teachers. Titled *Biofilms in Practice: A Hands-on Guide to Understanding Biofilms,* this curriculum focuses on the composition, concepts and importance of biofilms in the marine environment. "Biofilms are often a hard subject to conceptualize and understand because you can not see them," noted Mojica. "By designing user friendly lab experiments I hope to help teach students the importance of biofilms by showing them that it is often what you cannot see that leads to the wonderful things you can."

Within a two-year project timeline for classroom implementation, Kristina has identified the steps and review process necessary in making this a seamless addition to science programs within schools throughout Hawai'i.