Microbiome of a Boat

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Introduction

Bacteria and other microorganisms have colonized nearly every corner of the earth. These communities of microbes, known as microbiomes, affect everything from ecological processes to human health. Each microbiome’s bacterial makeup is as varied as the environment itself leading to a variety of community structures and survival techniques among the different populations. Notably, for aquatic bacteria, it is advantageous to be adhered to a surface rather than existing in suspension (Dunne 2002). Nutrients are more concentrated near a surface than in an aqueous medium and cells in close proximity to each other can readily exchange plasmids thereby increasing their resistance to antibiotics and overall genetic variation (Donlan 2002). When bacterial cells become encased in an extracellular matrix and begin adhering to each other, the conglomeration becomes known as a biofilm. Biofilms are often observed on implanted medical devices, medical equipment, water supply systems, and many other surfaces (Donlan 2002).

Biofilms in rivers are of particular interest due to their high level of diversity. Bacterial communities in rivers are generally very diverse because aquatic communities are mixed with communities found in runoff and soil in suspension (Lyautey 2005). Depending on the river and the surrounding area, bacteria from a variety of genera may be found in aquatic samples. While samples from larger rivers throughout the world have been analyzed for bacterial diversity (Cottrell 2005), none have been taken from smaller rivers, such as the Mystic or Charles River. River biofilms may form on anything from aquatic plants to discarded tires and soda bottles.

Due to their frequent immersion in rivers and other bodies of water, rowing shells are frequently colonized by riverine bacteria. Rowing teams employ various techniques to clean the surfaces of their shells prior to and following immersion in water, which helps to prevent buildup on the boat. Microbial buildup may decrease the speed a team can achieve (Haslbeck 1992), and cause the transfer of bacteria from one body of water to another. Popular cleaning techniques include the use of biodegradable soaps and dish soaps. The effectiveness of these techniques, however, is unknown. Knowing what types of bacteria are present in biofilms that form on rowing shells can help one to determine the best method of cleaning and also show if a particular technique is effective.

One way to determine the species of bacteria present in a sample is by matching the sequences from 16S rRNA genes to reference samples. The 16S rRNA gene, which codes for the RNA portion of the small subunit of the bacterial ribosome, is a gene that is present in all bacteria. Due to the functional stability of the highly-structured RNA the gene encodes, the mutation rate is low. There are, however, a variety of regions in the gene that may have many mutations, though these regions are unique to each species (Clarridge 2004). The 16S rRNA gene is approximately 1,550 bp long, although sequencing of the entire gene is generally not necessary unless one is describing a new species. Researchers have noted that the first 500 bp
may contain as much as 60% of the variability in a given species (Kattar et al. 2001). By using high-throughput sequencing technology, such as the MiSeq sequencer, one can identify the various species of bacteria in a sample both quickly and cost-effectively.

Objectives
The objectives of this study are two-fold. The MiSeq sequencing technology will be used to:

- Determine the species of bacteria that colonize submerged and unsubmerged surfaces of rowing shells
- Study the effects of current cleaning methods on existing bacterial populations

We hypothesize that bacteria will be found on all swabbed surfaces of rowing shells. Dawn soap and bleach will effectively reduce bacterial populations due to high concentrations of surfactants or the ability to denature proteins, respectively. Simple Green, while a popular option, does not contain ingredients that are known disinfectants so we hypothesize that it will not produce a significant change in bacterial populations. Because mechanical disruption of the biofilm may play a role, we will use cleansing with pure water as a negative control.

Methods
Prior to any sample swabs being taken, a protocol must be developed for collecting surface samples. While literature has suggested that using a swab soaked in buffer is more effective than a dry swab (Buttner et al. 2001), it is not known whether enough cells can be collected from a rowing shell on one swab alone. A BioSeq protocol used for swabbing the retroauricular crease in order to study the human microbiome will be modified and tested on various surfaces.

The initial step of the protocol, soaking a sterile swab in buffer solution, will remain the same. In order to test how many swabs are necessary to collect sufficient materials, one, two, and five swabs will be used to collect samples from a clearly defined area on a surface. The area will be swabbed in two perpendicular directions to maximize coverage. As a negative control, a single swab will be soaked in the buffer and then soaked in the extraction solution without taking a sample. As a positive control, a single swab will be used to sample the retroauricular crease, a surface known to have a variety of cells on it. The single and multiple swab trials will follow the normal protocol with the modification that all swabs for a single trial will be soaked in the same extraction solution. All three trials will then follow the rest of the BioSeq protocol for cell lysis and preparation for PCR. Following PCR1, the amount of DNA present in each sample will be quantified using the Bioanalyzer.

Once the optimal swabbing technique has been determined, samples can be collected from the rowing shells. Five locations (underside of boat, bow deck, rudder fin, inside of shoe, and end of tracks) will be swabbed following a shell’s normal amount of time in the water. After allowing the boat to dry for thirty minutes, swabs at analogous locations will be taken as controls to see the normal amount of change following removal from the water. In the next trial, boats
will be swabbed in the five test locations upon removal from the water but will then be cleaned using one of the test materials. Following the thirty minute waiting period, samples will be taken in analogous locations.

After collection, swabs will be brought back to the lab and the 16S rRNA gene will be amplified by direct PCR, sequenced, and metagenomic analysis will be performed on the resulting sequence. To determine the effects of the various cleaning techniques, a comparison will be done between the baseline and analogous post-cleaning samples following metagenomic analysis. Sample composition will be compared to identify any changes in specific populations within the communities as well as trends between sites.

References

Biography
Abby Candee, a recent graduate of Medford High School, will be attending Mount Holyoke College in the fall.