

## **Recovery and identification of marine microbes from Narragansett Bay and assessment of their potential for biofilm formation in single and mixed culture models.**

Anne Reid (Salve Regina University)

### **Project Location:**

Salve Regina University

### **Project Description:**

The recovery of marine microbes in pure culture remains a challenge, as these microbes are found in low abundance, do not grow readily on standard growth media, and often rely on interactions with other microbes for growth and viability. Studying the microbes found in Narragansett Bay will help us understand how microbial population vary in response to climate change, and may lead to the identification of biomarkers for monitoring the health of our local marine ecosystem.

During the SURF 2018 season, culture conditions (for instance filtration of seawater and plating on marine agar, or dilution to extinction in marine broth) were identified which resulted in recovery of marine microbes. While a number of isolates have been recovered, we will continue to sample water from various sites in Narragansett Bay and to recover additional microbes using these previously-established protocols. This continued sampling will enable us to assess stability of microbial populations in select areas over time, as well as expand the limited sampling range studied in year 1. These recovered microbes will be identified by PCR amplification of barcode genes (16S rRNA for bacteria, ITS for fungi) and Sanger sequencing. Stock cultures of these isolates will be preserved in glycerol, and these microbes will be assessed for their ability to form biofilms.

Biofilm formation will be assessed under aerobic and anaerobic conditions using a standard microtiter plate assay. Both single-species and mixed-species biofilms will be studied – selection of microbes for these assays will be guided by literature analysis of known or suspected cooperative interactions between microbes. Microbes in the biofilm will either be quantified indirectly by staining with crystal violet, or serially-diluted and directly enumerated. In addition to biofilm formation in polystyrene plates, biofilm formation will also be assessed on coupons of PMDS, a material to be used in the biosensors being developed by researchers in thrust 3. The PMDS coupons will also be imaged by confocal microscopy in order to understand the size, spread and architecture of the biofilms produced on this type of material. An understanding of how biofilms form and which microbes can contribute will provide us with information that can guide strategies to mitigate biofouling of the sensors.

It is well-understood that only a fraction of the microbes in a marine environment can be recovered in laboratory culture. As such, the microbes isolated above represent a small portion of the total microbial communities in the waters of Narragansett Bay. Next Generation Sequencing will be deployed to gain a broader understanding of the microbial communities in these waters, and to understand how our isolates fit into the larger network of microbes in these samples. Total genomic DNA will be isolated from seawater samples and the 16S rRNA barcode for bacteria will be amplified by PCR. DNA libraries will be prepared and analyzed by NGS on an Illumina MiSeq (housed at Salve Regina University). Data will be analyzed using an established QIIME2 pipeline, and the bacterial populations from different sample sites and different sampling seasons (samples collected during year 1 and 2) will be compared in order to gain a better understanding of the stability and composition of the Narragansett Bay microbial communities.

*This project involves **primarily lab or computer work***

**Required/preferred skills for student applicant:**

The following skills are preferred, but not required

- training will be provided to the successful candidate(s)
- preparation of agars and broths for bacterial culture
- aseptic technique and culture of microbes
- basic molecular biology skills (use of pipettor, agarose gel electrophoresis, PCR)

**Student transportation needed for project?**

Yes

- student will need to collect water samples at several sites over the summer (frequency: no more than once/week)
- if student does not have access to own vehicle, alternate arrangements can be made – a student should not be swayed from applying even if they do not have their own vehicle