Environmental Bacteriophage Detection on Coastal Carolina University Campus

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Introduction

The purpose of this study was to utilize bacteriophage as an environmental indicator of the presence of harmful bacteria in waterways on Coastal Carolina's campus and to identify bacteriophage that could be used to control bacterial blooms. Bacteriophages are viruses that infect bacteria. These viruses are found ubiquitously in the environment and are the most abundant organism on Earth. Eleven sites are designated for weekly sample collection. Water samples are filtered and amplified using strains of *E. coli B* and *E. coli K12* to allow potential viruses in the sample to proliferate to detectable levels. Plaque assays are used as a microbial screen for the presence of bacteriophage. Samples that test positively using the microbial test are analyzed through a molecular test using PCR and gene specific primers, which identify the viral families and confirm the presence of the desired bacteriophage. The results of this study illustrate the presence of bacteriophage on the Coastal Carolina's campus and the identification of at least one of the desired viral families.

Sample Sites

Eleven sample sites on Coastal Carolina's main campus were chosen. The experimental sample sites included both treated and untreated water bodies.

Figure 1: Satellite Map of CCU's Main Campus with Labelled Sample Site Locations

Methods

Collection: At 11 sites around main campus, water samples were collected weekly with the chance of collecting bacteriophages. pH and water temperature were measured to establish the ambient, surrounding environmental conditions.

Bacteriophage Alpha Test: The water samples were filtered to remove potential microbes and were amplified to allow potential bacteriophage to replicate to a detectable level. A microbial test was conducted to detect the presence of lytic bacteriophage. 2x LB agar plates served as a growth medium for the 2 different strains of *E. coli*. These plates were divided into quadrants following the bacterial inoculation. Three quadrats received the amplified site-specific sample while the remaining received the control. Any present phage will lyse the growing bacteria and leave a zone of lysis that will not have bacterial growth. This zone of clearing serves as an indication that lytic bacteriophages are present.

DNA Extraction: Samples positive for lytic bacteriophage undergo crude DNA extraction to prepare for a molecular test. Approximately 100 µL of supernatant was added to a microcentrifuge tube with 5 µL of proteinase K. The samples were put in a shaking incubator for 60 minutes at 38ºC and then were placed on a heat block for 5 minutes at 95ºC to deactivate the proteinase K and to encourage further lysing.

PCR Testing and Gel Electrophoresis: During the PCR, target viral genetic material was amplified using the T4 program. The amplified samples were run on a 2% agarose gel at 60 volts for 1 hour, and a 100 base pair DNA ladder served as the reference.

Results

Routine physicochemical measurements illustrate that the pH and water temperature were within suitable ranges. There was no statically significant difference between the average pH or average temperature (°F) across the experimental sample sites (p-value > 0.05).

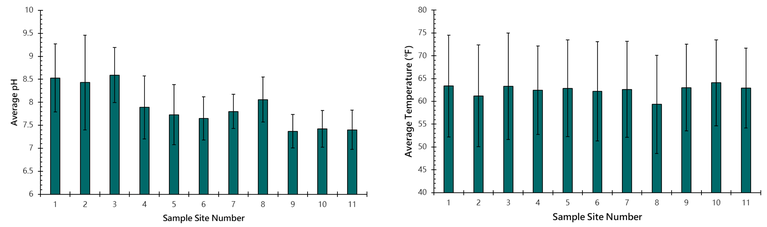

Figure 2: Physiochemical Measurements from Weekly Water Sampling
The error bars represent standard deviation.

The most positives were seen in sample sites 9, 10, 11, which compromise an untreated stream that runs through main campus. These sample sites are all connected, so they experience similar environmental influences.

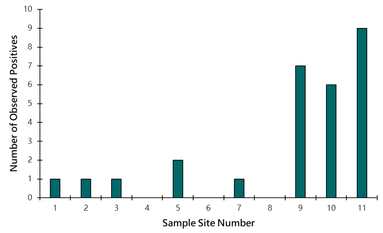

Figure 3: Number of Observed Bacteriophage Positives Seen Across Sample Sites

Of the positives observed, the most notable results were similar morphologies on plaque assays from amplified environmental samples collected on the same day from related sample sites. Also, the visually comparable results shown on the gel were interesting as they represent samples taken on three different collection days.

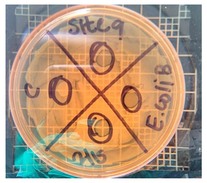

Figure 4: (2/15 Site 9) A positive microbial result and exemplifies common morphology

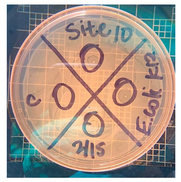

Figure 5: (2/15 Site 10) A positive microbial result and exemplifies common morphology

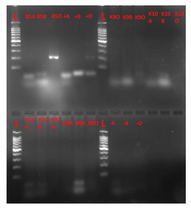

Figure 6: (10/26) A positive molecular PCR result of the O Primer Set

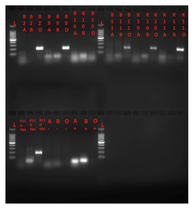

Figure 7: (2/15) A positive molecular PCR result of the O Primer Set

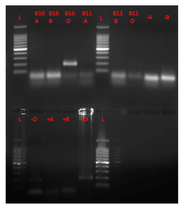

Figure 8: (3/15) A positive molecular PCR result of the O Primer Set

Organism	Sequence	Gene Target	Primer Name	Primer size	PCR Fragment size	
Collphage T2/T4	TGGCGCAGTAACTCAGATTG	Orf 23	Orf23 For	20bp	405	Orf
	GCACCAGCTTCCATTTGTTT		Orf23 Rev	20bp		Orf
Collphage T2/T4	CCCTGCGCCTTTCATAATAA	Orf 43	Orf43 For	20bp	198	Orf
	ATCGCAGGAACAGCTCCTAA		Orf43 Rev	20bp		Orf

Figure 9: Primer Set

Discussion

The purpose of this study was to utilize bacteriophage as an environmental indicator of the presence of harmful bacteria in waterways on Coastal Carolina's campus and to identify bacteriophage that could be used to control bacterial blooms. Bacteriophage was successfully found and identified in many of the 11 sample sites. Of the detected bacteriophage, the O primer viral family reoccurred most frequently. The most interesting results can be interpreted from the morphology and PCR of bacteriophage at sites 9, 10, and 11. The plaque assays displayed a strikingly similar morphology and the PCR subsequently showed comparable results for the sample site over time. This suggests that these observed viruses are either the same or very similar, and because of this, they attack similar strains of E. coli. For this trend to have been observed throughout the data collection, there must be a profuse source of this bacteria existing in proximity to the sample sites. This source must be experiencing longevity, which is allowing the methodology of using phage as an environmental indicator to be altered. This supports the hypothesis that phage can be used as a rapid and relatively inexpensive test to evaluate the health of an environment and to identify chronic environmental issues or contaminates.

Over past months of data collection, there have been very few PCR positive results, which further indicates a recent environmental change. The pH and temperature data have consistent averages indicating that these factors are not heavily influencing these blooms. Furthermore, these null environmental parameters likely suggest an unknown factor that could potentially be an infection within local wildlife, which would have the capability to affect the results.

Future Directions

As this project is scheduled to continue next school year, there are several key components that will be evaluated further based the observed results thus far. For example, expanding to more sample sites would provide a more complete look to the variation of water quality across space on CCU's main campus. Additionally, the project will grow in breadth as the goals expand to identifying and cultivating bacteriophage that infects specific strains of *E. coli*. The efficacy of the cultivated phage will be evaluated across several different strains of bacteria to test specificity. Using this knowledge, an environmentally friendly treatment for bacterial blooms on campus can be created using the samples collected across space and time.

Acknowledgements

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