Environmental Bacteriophage Presence in Drainage Ponds at Coastal Carolina University

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Research Questions

1. Are naturally occurring, lytic bacteriophages able to be detected on Coastal Carolina's Campus?
2. Can the environmental factors that influence their presence be identified?

Introduction

Bacteriophages are non-living viruses that infect bacteria, and they are found in abundance in every environment[1]. Similarly, coliphages are viruses that only infect coliform bacteria. Coliform bacteria and coliphages originate in the gut, so they both enter the environment by animals, humans, and municipal sewage[3]. Temperature is the most crucial factor in the proliferation of bacteriophages. At low temperatures, fewer phages can penetrate bacterial host cells; therefore, replication is hindered[4]. Likewise, lower than optimal temperatures can affect their bacterial host negatively[3]. Water temperatures greater 35°F and pH ranges outside of 6-8 better encourage the proliferation of coliphages specifically[2]. By better understanding coliphages, a natural solution can be found to suppress environmental bacterial blooms.

Sample Sites

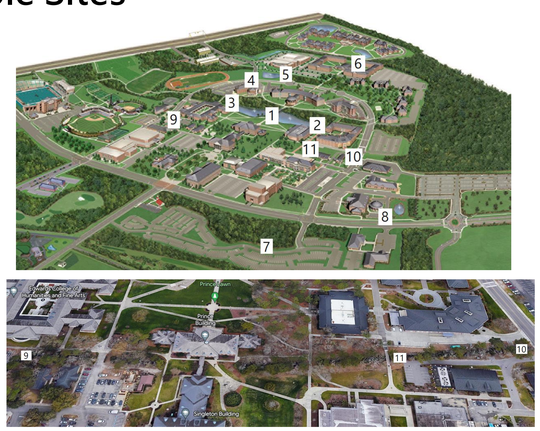

Figure 1: Maps of CCU's Main Campus with Labelled Sample Site Locations

Methods

Collection: Water samples were collected weekly with the chance of collecting bacteriophages, and the environmental parameters were measured.

Bacteriophage Plaque Assays: The water samples were filtered and amplified to allow potential bacteriophages to replicate to a detectable level. As a microbial test, plaque assays were conducted to detect the presence of lytic bacteriophages. Any present phage would leave a zone of lysis on the plates.

DNA Extraction: Samples positive for lytic bacteriophage undergo crude piece DNA extraction to prepare for a molecular test.

PCR Testing and Gel Electrophoresis: During the Polymerase Chain Reaction (PCR), target viral genetic material was amplified using three primer sequences.

CPA

Family	Virus	Base Pair Size
Siphoviridae	HK	177
Podoviridae	933	488
Myoviridae	T4	704
Microviridae		1039
Podoviridae	K1F	2110

CPB

Family	Virus	Base Pair Size
Myoviridae	Mu	177
Siphoviridae	1	307
Siphoviridae	JK	878
Podoviridae	N4	2285

CPO

Family	Virus	Base Pair Size
Orf 43	T-even	198
Orf 23	T-even	405

Figure 2: Primer Sequences Used in PCR

Results

There was no statically significant difference between the average pH or average temperature (°F) across the experimental sample sites (p-values > 0.05). Precipitation had no correlation with the frequency of positive bacteriophage results (r ≈ 0). Although, water temperature (°F) and pH did vary significantly across the collection dates (p-values < 0.05).

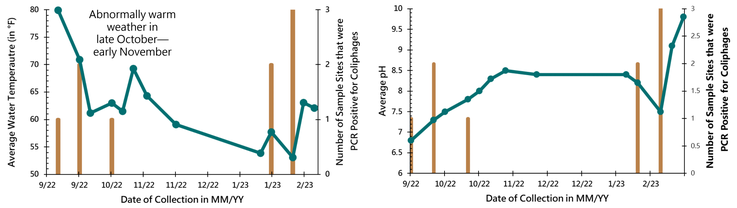

Figure 3: Physiochemical Measurements from Weekly Water Sampling Compared to the Presence of Coliphages

Most PCR positives were seen in sample sites 9, 10, 11, which compromise an untreated drainage pond that runs through main campus. These sample sites are connected, so they experience similar environmental influences, too.

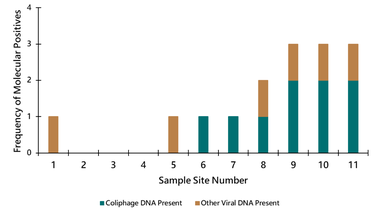

Figure 4: Number of Observed Bacteriophage Positives Seen Across Sample Sites

Of the positives observed, the most notable result was the successful identification of one viral family, *Siphoviridae*, in the environmental samples. Sample sites positive for this viral family had varying visual indications on the plaque assays. In Figures 6-8, the present coliphages were outcompeted on the plaque assays resulting in non-determinant results. Reflected in Figures 10-12, sites PCR positive for *Siphoviridae* contained amplified DNA strands indicative of belonging to that family, which were facilitated by Primers CPA and/ or CPB and CPO. Also, it is important to recognize samples positive for *Siphoviridae* were collected at various points in the year but under similar environmental conditions.

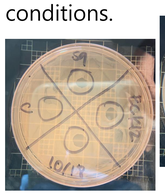

Figure 5: (10/18) Site 9 plaque assay positive for lytic phage

Figure 6: (1/30) Site 6 plaque assay non-determinant

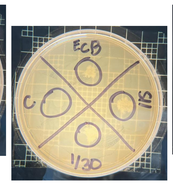

Figure 7: (1/30) Site 11 plaque assay non-determinant

Figure 8: (2/13) Site 7 plaque assay non-determinant

Figure 9: (2/13) Site 9 plaque assay positive for lytic phage

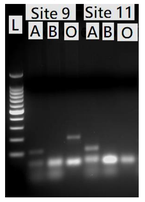

Figure 10: (10/18) Site 9 positive for *Siphoviridae*

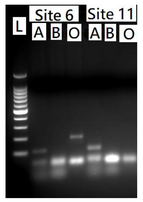

Figure 11: (1/30) Sites 6 and 11 positive for *Siphoviridae*

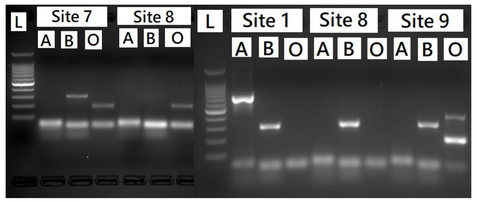

Figure 12: (2/13) Sites 7 and 9 positive for *Siphoviridae*

Discussion

The purpose of this study was to detect naturally occurring coliphages and to identify the environmental influences that encourage their presence. Coliphages and other viruses were successfully found and identified in many of the 11 sample sites. Of the detected coliphages, the viral family *Siphoviridae* was identified successfully by the molecular test. This viral family was found at four sample sites across separate collection dates. Three of those four sample sites represent untreated drainage ponds on CCU's campus. Additionally, the plaque assays completed for sites visually differed. While a couple plates displayed appropriate results indicating lytic activity, most appeared to be non-determinant, meaning something outcompeted the present phage. Because this viral family was found in environmental samples across several collection dates, there must be a profuse source of its bacterial host present in proximity to the sample sites.

The ambient environmental conditions, measured by water temperature and pH, significantly fluctuated across the collection period. As pictured in Figure 3, the presence of bacteriophages is seasonal. In November through early January, no coliphages were identified in the sample sites by both a microbial and a molecular test. This supports the hypothesis that coliphages proliferate in warmer water temperatures. However, the hypothesis that coliphages exist better outside of the 6-8 pH range was not supported by the results. In both neutral and acidic environments, coliphages were found. It is important to recognize that most coliphages and other viruses existed in the untreated drainage ponds on CCU's campus. These environments are more likely to experience long-term, persistent pollution, so bacteria can grow uninterrupted, which naturally warrants the presence of bacteriophages.

Future Directions

As this project is continuing over the summer and next school year, there are several key components that will be evaluated further based the observed results. For example, expanding to more sample sites that represent untreated environments on CCU's campus would provide a more complete look to the variation among untreated water on campus. Also, a statistical approach of different environmental factors such as precipitation, water temperature, air temperature, and ambient weather conditions will be employed as this project continues. This approach will help identify the correlation between external, environmental factors and the presence of coliphages.

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SC INBRE
South Carolina IDeA Networks of Biomedical Research Excellence

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