Searching for Atlanticus: Isolating Bacteriophages Infecting *Marinobacter*

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Abstract

Bacteriophages (phages) are viruses that infect bacteria. They have a considerable effect on ecosystems (Focardi et al., 2020). Scientists believe phages are present virtually everywhere, although marine bacteriophages have remained largely undiscovered and understudied. Using ocean water samples from various parts of the United States (Figure 1), protocols adapted from the SEA-PHAGES program (site Discovery Guide), and a flocculation protocol (John, et al 2011) we are attempting to isolate bacteriophages that infect two species of marine bacteria, *Marinobacter hydrocarbonoclasticus* and *Marinobacter atlanticus*.

Introduction

Bacteriophages [Phages] are viruses that can be found in differing environments and infect specific bacteria. Phages can be found in a variety of environments, including in the ocean (Sreekumar, 2020; Zhu et al., 2018). While phages are abundant in marine environments, there is not much documentation about marine phages. To date, there are no documented phages that infect *Marinobacter hydrocarbonoclasticus* or *Marinobacter atlanticus*, which are two species of a marine bacterial genus *Marinobacter*. To assist a researcher at the Naval Research Institute this project was a search for bacteriophages that infect *M. hydrocarbonoclasticus* and *M. atlanticus*.

M. hydrocarbonoclasticus is a gram-negative, rod-shaped, aerobic bacteria that exhibits extreme halotolerance, hydrocarbon degradation, and belongs to phylum Proteobacteria (Gauthier, et al., 1992). It was first isolated in the Mediterranean Sea; however, it can be found in diverse oceanic environments. Similar to *M. hydrocarbonoclasticus*, *M. atlanticus* is a rod-shaped bacteria that is halophilic (Wang et al., 2015). It was originally discovered off of the coast of New Jersey but can also be found in numerous oceanic environments.

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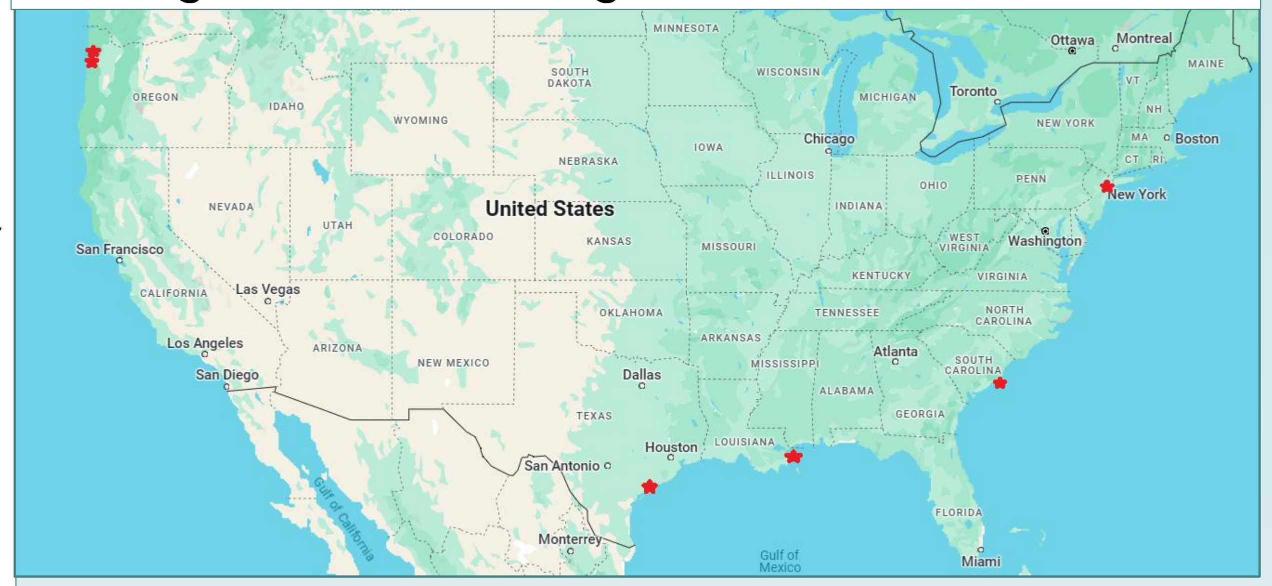
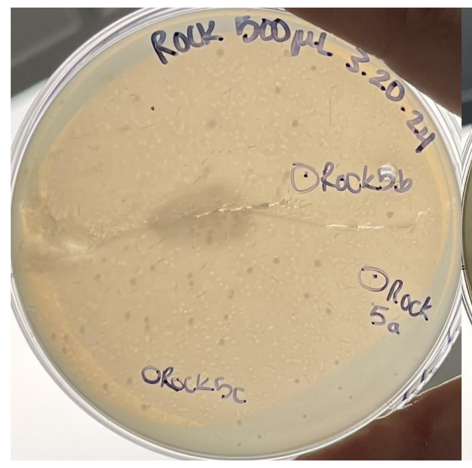
Methodology

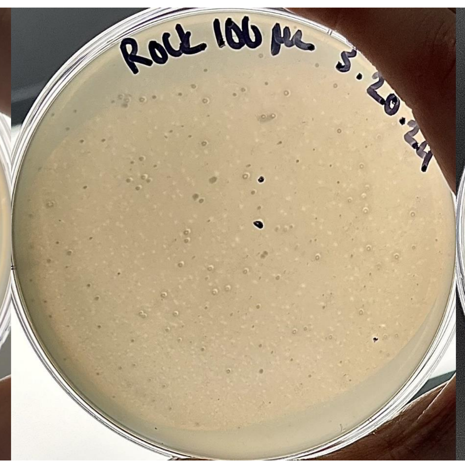
Samples were isolated from the regions marked with red stars on figure 1. Samples were filtered through a .2 micron filter and kept in at 4℃ until beginning the enrichment protocol. Enrichment consisted of spiking filtered samples with 250μL of either *M. hydrocarbonoclasticus* or *M. atlanticus* and marine broth mix and were kept in a shaking incubator at 34-37℃ for several days. The next step was to conduct plaque assays which involved taking the enriched samples, spiking with 250μL of *Marinobacter*, incubating for 20 minutes, then plating with 4mL of Marine top agar and placing plates into the incubator for 2-3 days. If plaques were seen on these plates after the incubation period, these plaques were "picked" and used in a serial dilution on a Spot Test plate. This consisted of creating a 0 to -10 dilution of the picked plaque and pipetting 5μL of the dilutions onto a plate with a *Marinobacter* top lawn. The purpose of conducting spot tests is to determine with more confidence if the plaques seen in the plaque assays were actually due to the presence of a virus. . The next step is to isolate the phage from the original plaque that was seen on the plaque assay plates. This step of the process is where this currently is. Another method of phage isolation from sea water is the flocculation protocol from John et al., 2011. This involved filtering sea water, adding aa iron solution to precipitate the viruses present, and collecting the precipitate by filtering the sample through a .8 micron filter. The precipitate viruses could then be resuspended and further isolated.

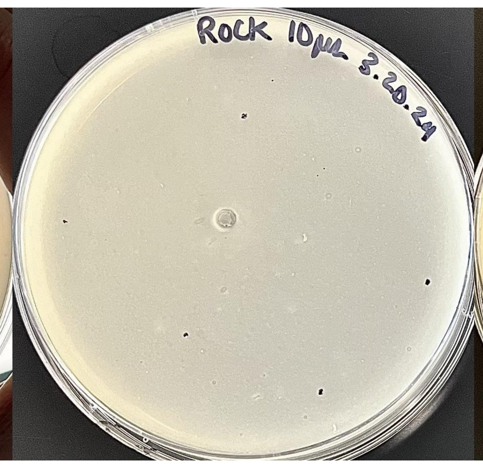
Figure 1: This map shows where each tested sample has originated from using red stars.



Results


Rock 500μL

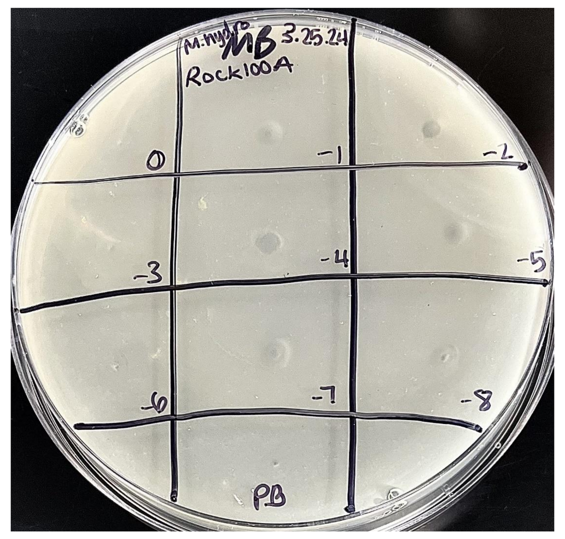

Rock 100μL

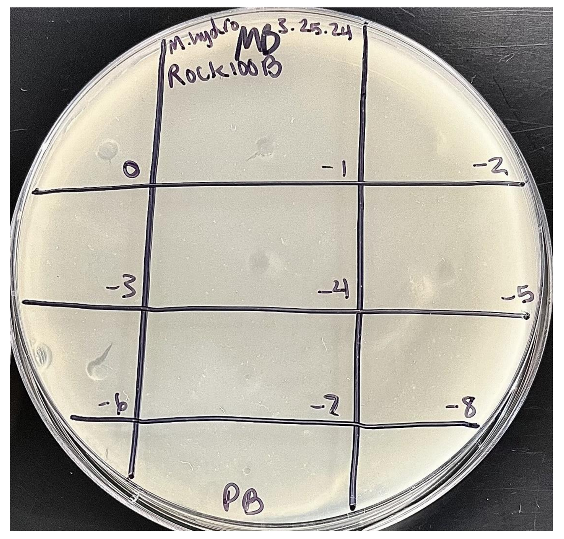

Rock 10μL

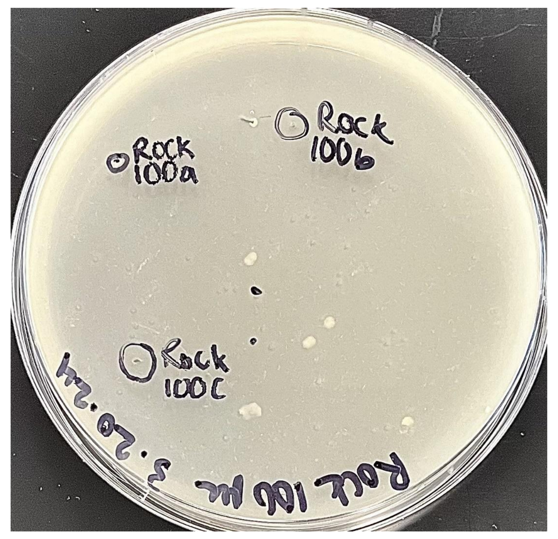

Negative Control

To the left are pictures of a successful plaque assay. Phages were present in all 3 dilutions. These samples came from Rockaway Beach, OR. The plate labeled Rock100 was chosen to continue in the isolation process.

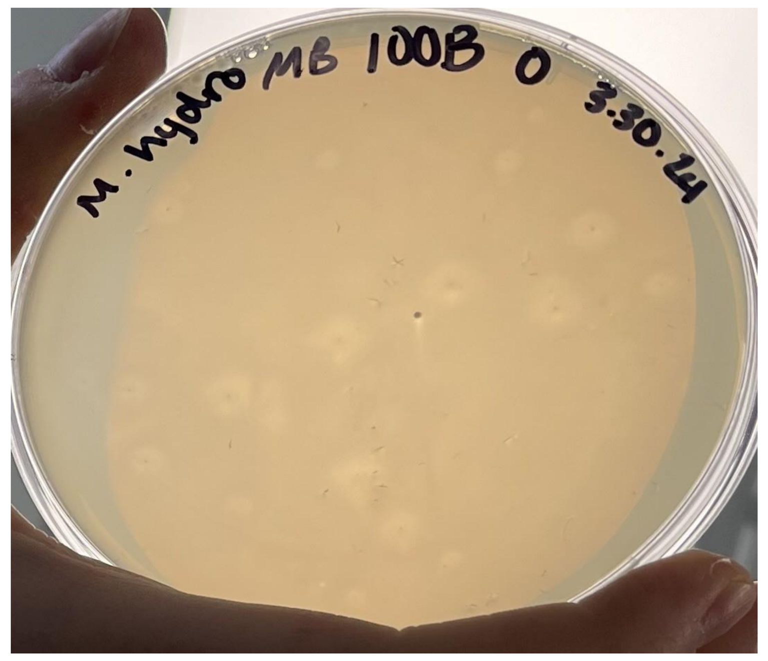
The plaques found in these samples are very small, round, and extremely clear. While holding a plate up to a ceiling light (as in the Rock 500 and 100 sample pictures) you can see the plaques as tiny white circles amongst the cloudy bacterial lawn. *M. hydrocarbonoclasticus as* the bacterial lawn.


Rock100


Rock100B


Original Rock 100μL

Above are two examples of a successful Spot Test. Each phage was 'picked' from the original Rock100μL plate and diluted to 10^{-8}. Seeing clearing in all grids is a good sign that a phage specific for the bacteria on the lawn is present. In this case, this was further evidence that I found a phage specific for *M. hydrocarbonoclasticus*. To the far right is the original Rock100μL from the plaque assay with the isolated plaques labeled.



Above is an example of the first successful phage isolation experiment. Using the same plaque as the Rock 100B spot test, several dilutions were made to begin isolating a single virus from any others that may have been present in the plaque.

Conclusion

While bacteriophages are present in abundance in ocean samples, (Sreekumar, 2020; Zhu et al., 2018) it has been challenging to isolate phages that infect specific species of Marinobacter. Throughout the past semester, I have found a virus that infects M. hydrocarbonoclasticus. I am now on the third and final round of phage isolation and one step closer to extracting and replicating the phage's DNA. As of now, I have been unable to discover a phage that infects M. atlanticus. However, as there are still 4 weeks left in the semester, I have time (and plenty of samples) to keep looking.

References

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