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# Making Waves in Phage Research: Pacific's Journey through Discovery, Experimentation, and Clustering

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# Making Waves in Phage Research: Pacific's Journey Through Discovery, Experimentation, and Clustering

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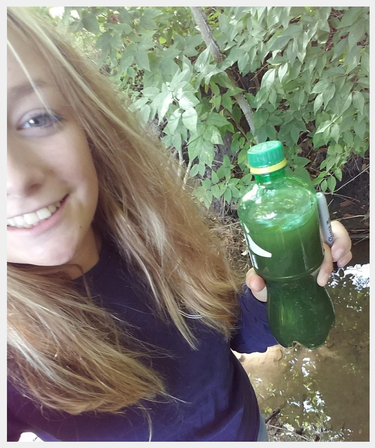
## Abstract

Phages are a class of viruses that infect bacterial cells. The purpose of the research is to locate and discover new phages to gain a better understanding of the vastly unknown area of microbiology. We began our research by first hunting for phages. They were amplified using sample enrichment, had their DNA extracted, then had their genome sequenced. In order to group the phages into clusters of known type, we utilized tests such as: lysogen testing, host range testing, and transmission electron microscopy (TEM). Our research focused on the isolation and sequencing of a cluster D phage known as Pacific. By annotating and publishing Pacific's genome, scientists are able to better understand overall phage diversity and evolution.

## Isolation of Phage

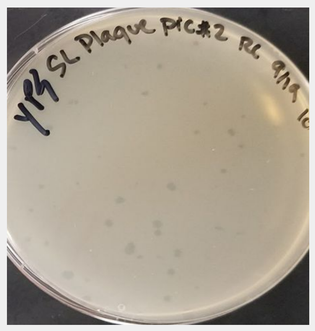


Date Isolated: 08/30/17

Type of Sample: Water

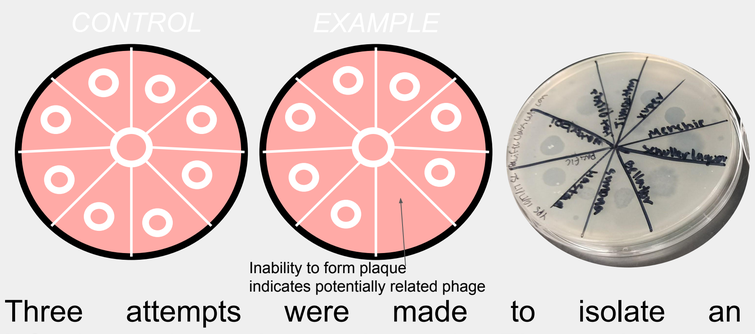
Description of Location: Taken from stagnant area of a creek in Mahomet, Illinois. The air temperature was 77 degrees. Lot of plant and animal life in water.


## Plaque Morphology



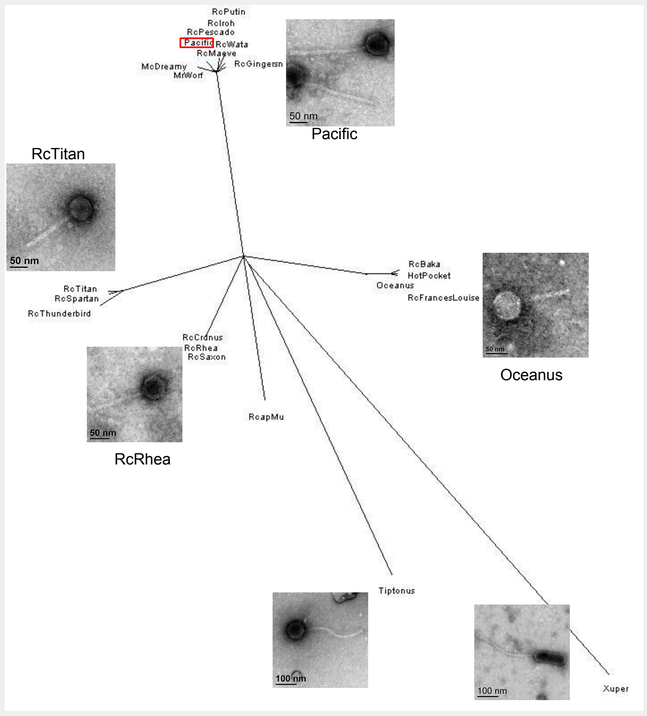

Pacific's plaques are "bulls-eye" shaped (clear inside, cloudy outside). This indicates that Pacific is a temperate phage (able to perform both the lytic and lysogenic cycle).

## Pacific Lysogen Testing Results



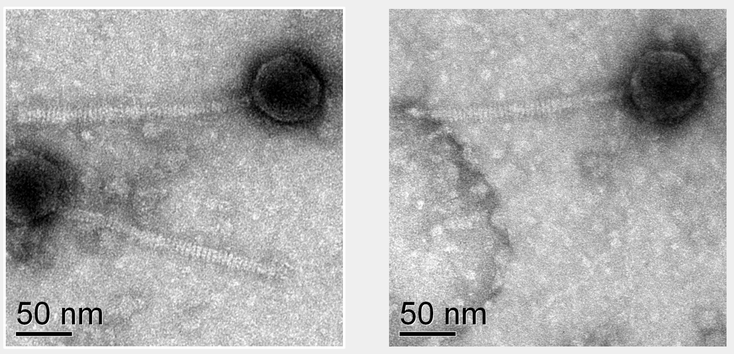

Three attempts were made to isolate an infection-resistant lysogenic strain. The strains were tested against the other isolated phages. The ability of the 8 isolated phages to infect the lysogen were compared to the ability of the phages to infect the original host. Pacific, RcBaka, Hotpocket, and none of the past C or D phages were able to produce plaques on Pacific's Lysogen, this could indicate a potential relationship between Pacific and these phages.

## Pham Tree




After sequencing phages, they can be placed into different "clusters". They are placed into the same cluster as another phage if more than 50% of their genomes align. Pacific is in the D cluster, which already has 8 other phages in it.

## Transmission Electron Microscopy (TEM)




TEM analysis revealed Pacific's structure. Pacific was determined to be a *Siphoviridae* (a DNA filled capsid with a long, noncontractile, thin tail, which is often flexible). Pacific had a capsid diameter of 68.33 nm and a tail length of 195 nm. In comparison to other phages of the same cluster, these measurements were around the average measurements: the average tail length of cluster D phages is 201.35 nm, and the average capsid diameter is 66.79 nm.

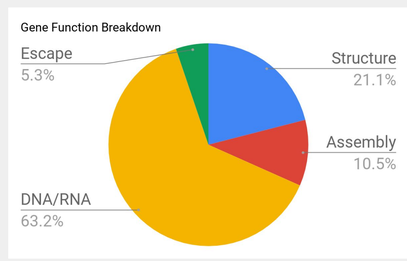
## Host Range Test

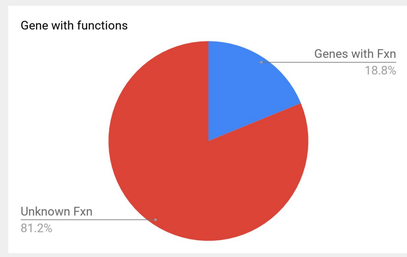
| | YW1 | YW2 | B6 | B10 | St. Louis | 37B4 | Iona |
|---|---|---|---|---|---|---|---|
| Xuper | + | + | + | + | + | X | X |
| Schuyler Lagoon | + | + | + | + | X | X | X |
| Waterboi | + | + | X | X | + | X | X |
| Jimouthy | + | + | + | + | X | X | X |
| Menchie | + | + | + | + | X | X | X |
| RcBaka | + | X | X | X | X | X | X |
| Hot pocket | + | X | X | X | X | X | X |
| Hoffback | + | + | + | + | X | X | X |
| Pacific | + | + | + | + | + | X | X |
| McDreamy | + | X | X | + | X | X | X |
| Iroh | + | + | + | + | + | X | X |
| Wata | + | + | + | + | + | X | X |
| Putin | + | + | + | + | + | X | X |
| Maeve | + | + | + | + | + | X | X |
| Pescado | + | + | + | X | X | X | X |
| Gingersnap | + | X | X | X | X | X | X |
| MrWorf | + | X | X | + | X | X | X |

KEY: Formed a Plaque  Did not form a plaque

The purpose of testing the host range is to see which strains of *R. capsulatus* various phages can infect. Due to mosaicism, some phages have parts of bacteria DNA in their genome that causes them to be able to infect that particular strain. The trends that are seen can typically be traced back to which cluster the phages are in.

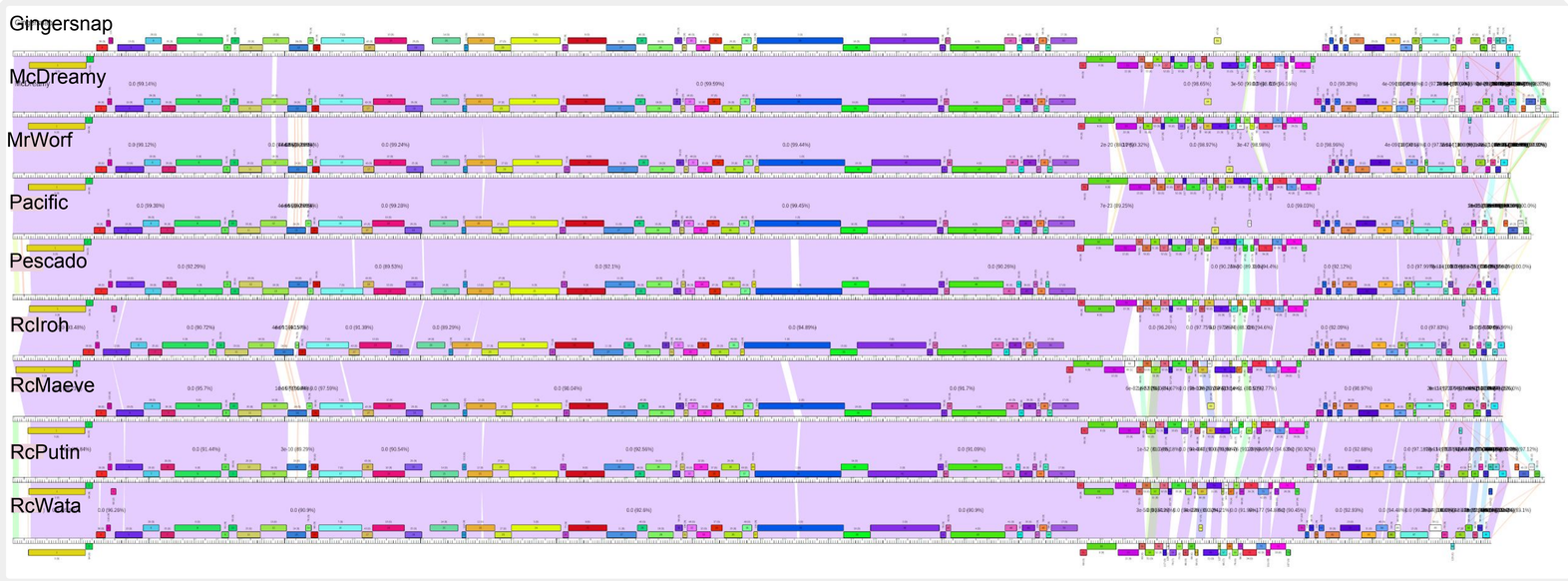
## Genome Analysis




After the genome was annotated and functions were determined, it was discovered that only ~19% of the genes had known functions. Of these genes, 63% of genes were DNA/RNA related, 11% were assembly related, 21% were structural, and 5% were escape related .

## Genome Map




The genomes of other cluster D phages were compared to Pacific. The colored cells represent genes that are shared among multiple phages. Areas in light purple identify high nucleotide identity between neighboring genomes, while regions in white indicate regions that are different.