



# VCU

Virginia Commonwealth University  
**VCU Scholars Compass**

---

Undergraduate Research Posters

Undergraduate Research Opportunities Program

---

2017

## Isolation of a Novel Phage OTooleKemple52

Thomas O. Raymond  
*Virginia Commonwealth University*

Follow this and additional works at: <https://scholarscompass.vcu.edu/uressposters>

 Part of the [Bioinformatics Commons](#)

© The Author(s)

---

### Downloaded from

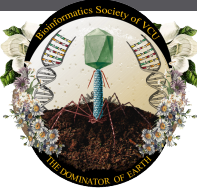
Raymond, Thomas O., "Isolation of a Novel Phage OTooleKemple52" (2017). *Undergraduate Research Posters*. Poster 228.  
<https://scholarscompass.vcu.edu/uressposters/228>

This Book is brought to you for free and open access by the Undergraduate Research Opportunities Program at VCU Scholars Compass. It has been accepted for inclusion in Undergraduate Research Posters by an authorized administrator of VCU Scholars Compass. For more information, please contact [libcompass@vcu.edu](mailto:libcompass@vcu.edu).



# Isolation of a Novel Phage OTooleKemple52

Thomas Raymond and Allison Johnson  
Center for the Study of Biological Complexity, VCU, Richmond, VA



### Introduction:

A bacteriophage is a virus capable of infecting bacteria like ubiquitous soil-dwelling genus *Bacillus*. Within the *Bacillus* genus, there is the "ACT family" made up of *B. thuringiensis*, *B. cereus*, and *B. anthracis*, which are highly related but with different pathogenic characteristics. Because of this, phages isolated using a species in this group may have a broad host range encompassing several species from *Bacillus*. Since *B. cereus* and *B. anthracis* can result in mild to fatal sickness in humans, the non-pathogenic *B. thuringiensis* kurstaki was used to discover and characterize novel phages.

After isolation, the usage of genomic analysis in phage characterization is key to discovering more about the phage. By looking at predicted proteins and genomes, researchers can further document interrelatedness of the known phages.

Through studying phages we can work to better understand both phage diversity and the interrelatedness of the *Bacillus* genus. The benefits of studying bacteriophages have reaches from environmental to medical significance because of the ubiquitous and pathogenic characteristics of the host bacteria.

### Methods:

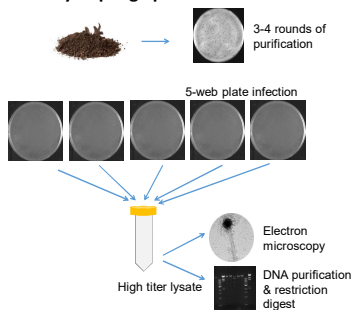
**Phage Discovery and Characterization:**  
The phage OTooleKemple52 was isolated from a soil sample collected from Chesapeake, VA using "soil enrichment." Soil enrichment involved putting a small amount of soil into a mixture of trypticase soy broth (a growth medium for bacteria) and *Bacillus thuringiensis*. The goal of enrichment was for the phage to infect bacteria, which would increase phage concentration and thus made infection more likely when plating. A phage plaque was observed from this enrichment infection upon plating.

The phage population was then purified until the morphology of the phage plaques was consistent (3 mm diameter with pinpoint clear centers). A large volume of phage stock (high titer lysate, HTL) was collected and then used to obtain purified DNA for gel electrophoresis and genome sequencing. Additionally, an HTL sample was stained with 1% uranyl acetate and imaged using transmission electron microscopy to determine morphology. The phage was tested for host range by spotting HTL into agar plates with various species of *Bacillus*.

### Phage Genome Sequencing and Bioinformatics:

The sequenced genome was annotated using DNAMaster. GeneMark and Glimmer were used to determine likely reading frames. Predicted proteins were submitted to NCBI's BLAST program to determine if they had segments that were present in conserved protein domains.

### Summary of phage purification and characterization



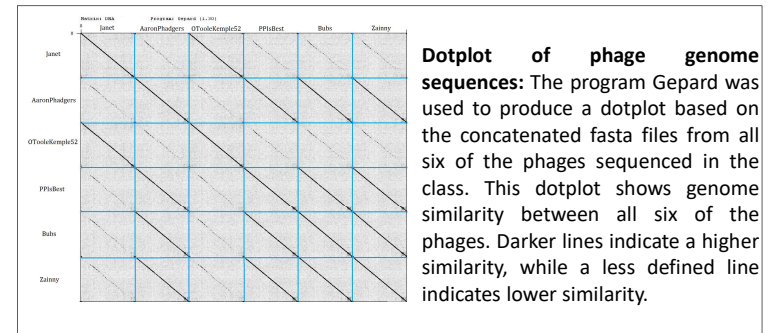
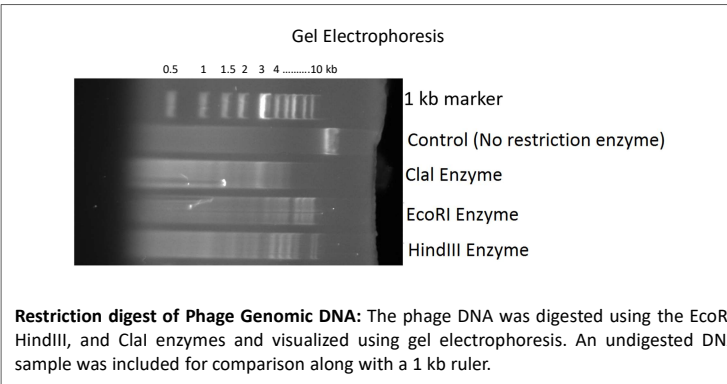
Soil Collection Date	Soil Origin	Plate image	TEM image
August 26 <sup>th</sup> , 2016	Chesapeake, VA		

Plaques: Pinpoint, 3mm  
Phage: myoviridae, head diameter of 80 nm, tail length of 200 nm.

Bacteria	Plaque Type
<i>B. thuringiensis</i> Kurstaki	clear
<i>B. thuringiensis</i> 350	clear
<i>B. thuringiensis</i> Konkukian	-
<i>B. thuringiensis</i> Israelensis	turbid
<i>B. cereus</i> T strain	turbid
<i>B. anthracis</i> Δ sterne	turbid
<i>B. cereus</i> 23857	turbid
<i>B. pumilis</i> ATCC 7061	-
<i>B. pumilis</i> SAFR32	-
Total # strongly infected	2

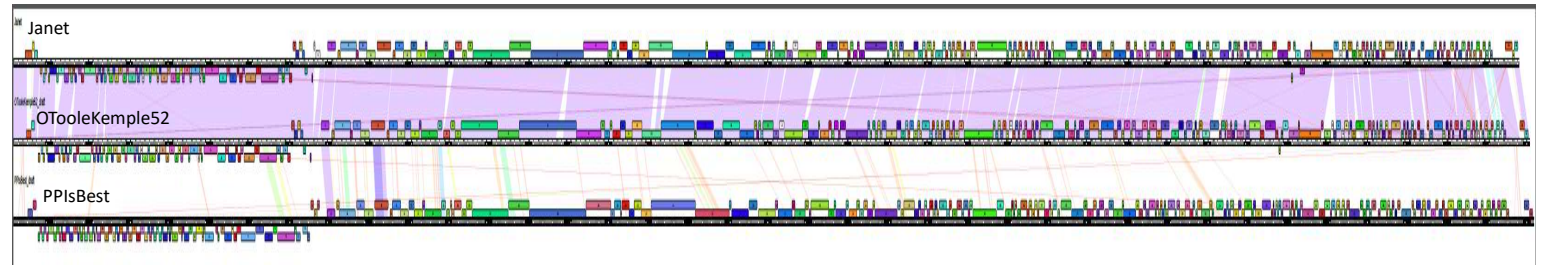
Host range testing: Testing was done to determine whether OTooleKemple52 had a broad or narrow host range. Clear plaques, suggesting a strong ability to infect, were only observed on two strains of *thuringiensis*. The ability to infect *B. cereus* T strain was explored further through additional plating, but infection was unsuccessful. Below, bacterial growth is visible where *B. cereus* and OTooleKemple52 were plated.

PHAGE GENOME CHARACTERIZATION					
Phage Name	Genome Length (bp)	Number of Genes	Number of tRNA's	Terminal Repeats (bp)	Genes with Predicted Function
OTooleKemple52	161,807	298	7	2426	55



### Genome map comparison of *Bacillus* phages OTooleKemple52, PPIsBest, and Janet

Comparisons were done between OTooleKemple52 and two other *B. thuringiensis* phages, using a comparative genome mapping tool Phamerator. Janet and PPIsBest. Janet is very similar to OTooleKemple52 in its proteins, while PPIsBest has far less similarity. Both phages were isolated by other students in the class. The purple sections on the map indicate regions of high nucleotide sequence similarity. Colored rectangles represent proteins and the color/order of the rectangle represent proteins in the same protein family. Genome similarity between OTooleKemple 52 and Janet is also observed in the dot plot.



### Acknowledgements:

VCU Life Sciences and the HHMI SEA-PHAGES program, Sequencing provided by the Pittsburgh Bacteriophage Institute, TEM provided by University of Mary Washington. Additional phages discovered by: Zainab Gbadamosi (Zainny), Brenna Kent (Janet), Rahul Warriar (AaronPhadgers), Erin Cochran (Bubs), and Nashwan Farooque (PPIsBest). If you're interested in bringing this program to your school, information can be found at [seaphages.org](http://seaphages.org)