Genomic and Biological Characterization of Mycobacteriophages Geralt and Phrodobaggins

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Abstract

In 2016, Drexel University completed its first year in the SEA PHAGES program. The first cohort of Drexel undergraduates captured and isolated 47 phages that infect *Mycobacterium smegmatis* mc^2 155. Out of the 47 phages, the genomes of eight phages have been sequenced. Of these eight sequenced phages, four belong to Cluster B, one belongs to Cluster C, two belong to Cluster F and one belongs to Cluster J. All students utilized electron microscopy to visualize their phages and document phage morphology, such as capsid head size and tail length. Several independent student experiments yielded interesting results this year. Students researched how infection conditions such as ion concentrations, pH and temperature affect their phage MOI. One compelling experiment examined the phylogenetic relationship of different clusters of phages. Two Drexel students, Christian Schill and Shayna Singh, created a phylogenetic tree based on the tail length of several different phages and found that length of the putative *tape measure* gene seems to be a predictor of evolutionary relationships between the phages being investigated. Several phages were tested for their ability to form lysogens. The similarity of lysogens from other phages was tested using the immunity assay. While many of the phages isolated in the class were tested for lysogeny, two phages, Geralt and PhrodoBaggins, isolated by Viktor Evtimov and Sravya Koduri respectively were also sequenced. The temparate phage Geralt readily integrated within *M. smegmatis*, but PhrodoBaggins was a purely a lytic phage. This finding was in agreement with the phage genomes as only Geralt has an *integrase* gene.

Temperate Bacteriophage Geralt: Lysogeny and Immunity

- Geralt is an F cluster Siphoviridae mycobacteriophage, isolated by Viktor Evtimov.
- Geralt's genome has an *integrase* gene and, therefore, can incorporate itself into the *M. smegmatis* genome. PhrodoBaggins was unable to form a lysogen and DNA analysis confirms the lack of an *integrase* gene.
- The putative lysogen isolated from the turbid mesa regions of Geralt plaques, Geralt- infected *M.smeg* was purified on 7H10 plates.
- Once the Geralt lysogen had been isolated, a liquid culture was established.
- As expected with a true lysogen, no lysis (and, therefore, no superinfection) occurred when the Geralt lysogen was plated with Geralt lysate. The Geralt prophage repressed the ability of the Geralt phage to infect the lysogen.
- When phage Superphikiman (Cluster J) was allowed to infect the Geralt lysogen, lysis occurred. This confirms that Superphikiman is not closely related to Geralt.

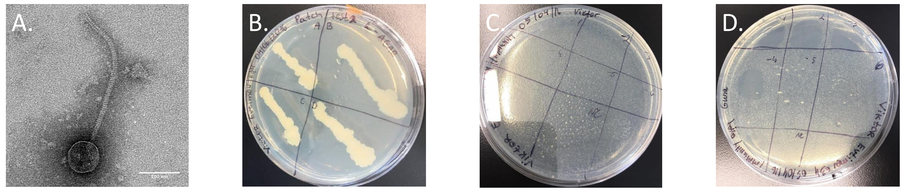
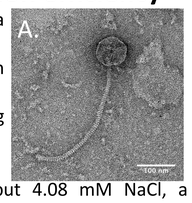


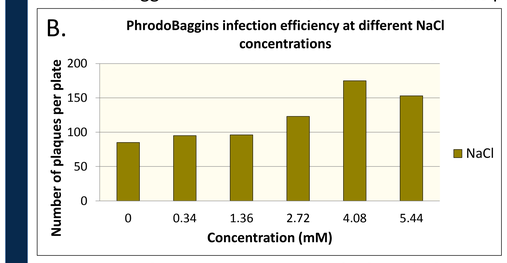
Figure 2: Characterization of Geralt as a temperate phage.

A) Electron micrograph of Geralt. The tail is 340.5 nm long and capsid is 76.5 nm in diameter. Scale bar 100 nm. B) Geralt Lysogen Purification on 7H10. C) Geralt does not lyse the Geralt-integrated lysogen verifying stable integration. D) Superphikiman, confirmed later to be a Cluster J phage, lyses the Geralt-integrated lysogen confirming a lack of relatedness between Geralt and Superphikiman.

Lytic Bacteriophage PhrodoBaggins: Characterizing infection efficiency

- PhrodoBaggins is a B cluster Siphoviridae mycobacteriophage isolated by Sravya Koduri
- Ion concentration and chemistry in phage buffer can affect adsorption and infection of bacteriophages.[1]
- Phrodobaggins infection was tested in 1) phage buffer without ions, 2) increasing concentrations of $CaCl_2$, $MgCl_2$, $BaCl_2$, and NaCl.
- Our results in Figure 3B and 3C show that Phrodobaggins exhibited the highest infection efficiency with 0.05 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.6 mM $BaCl_2$, but 4.08 mM NaCl, a concentration 6 times higher than the standard concentration of NaCl found in phage buffer. PhrodoBaggins was able to infect without ions in the phage buffer.





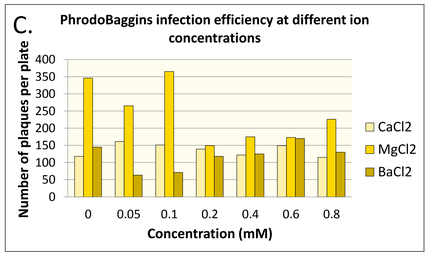


Figure 3: Characterization of Phrodobaggins as a lytic phage.

A) Electron micrograph of Phrodobaggins. The tail is 379.49 nm long and capsid is 76.7 nm in diameter. Scale bar 100 nm. B) PhrodoBaggins was incubated in phage buffer with a range of NaCl concentration with *Mycobacterium smegmatis* for 20 minutes and then plated using standard procedures and incubated at 37°C for 2 days. Plaque numbers were counted and are depicted. C) PhrodoBaggins was incubated in phage buffer with a range of $CaCl_2$, $MgCl_2$, and $BaCl_2$ concentrations and plated as in B.

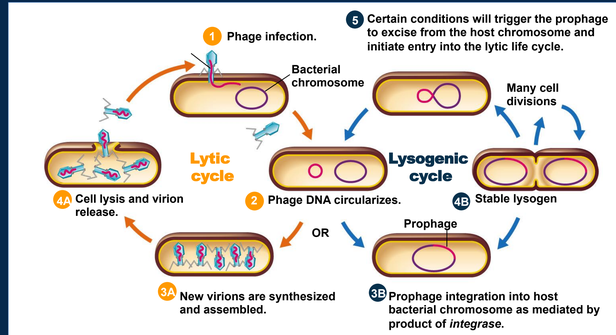


Figure 1: The bacteriophage life cycle.

Bacteriophage have two different life cycle phases. The lytic cycle mediates the production of new phage virions by capitalizing on the host replication machinery. The lysogenic cycle mediates a stable integration of the prophage that will be replicated as part of the host genome until an event triggers the re-entry of the prophage into the lytic cycle. A **lytic** phage, like **Phrodobaggins**, can only participate in the lytic life cycle, but a **temperate** phage, like Geralt, has the ability to undergo both cycles. Image modified from *Pearson Education*.

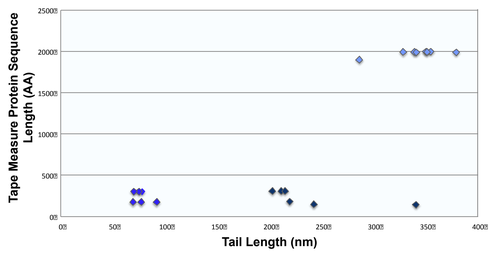
- Bacteriophage that infect *Mycobacterium smegmatis mc^{2}155* can be either lytic or temperate. A temperate phage can stably integrate into the host cell through activation of the *integrase* product that mediates homologous recombination of the host and phage genome.
- Drexel's first SEA-PHAGES cohort identified 47 different bacteriophage. 8 were sequenced and 5 were annotated as part of the *in silico* portion of the course.
- **In the Spring term, students were allowed to design and implement their own experiments. This poster reflects the work of several independent projects.**

Predicting bacteriophage cluster using *tape measure* protein and tail length

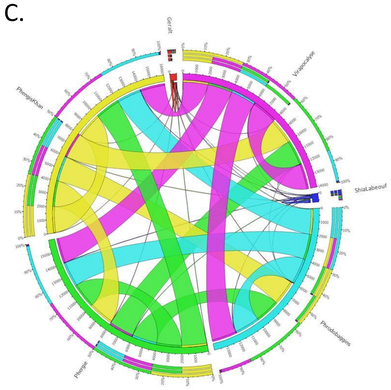
A.

Cluster	Location of Origin	Phage Name	Gene #	Tail Length (nm)	Tape Measure Protein Sequence Length (AA)
B	Philadelphia, PA	Phergie	27	350.0	1998
	Philadelphia, PA	PhengisKhan	26	354.6	1999
	Philadelphia, PA	Phrodobaggins	27	379.0	1991
	Philadelphia, PA	Virapocalypse	28	351.0	1992
	Santa Cruz, CA	Dori	24	286.3	1901
	Boulder, CO	Newman	28	339.1	1992
	Maple Grove, MN	Pipsqueak	28	328.3	1992
	Kenosha, WI	Squid	28	340.8	1991
C	Durban, South Africa	Dandelion	135	91.9	175
	Aledo, TX	Alice	124	78.0	302
	Bloomington, IL	Shrimp	131	75.0	302
	Mechanicsville, VA	Wally	130	70.2	302
	Grand Rapids, MI	Ava3	131	77.2	175
	Philadelphia, PA	ShiaLabeouf	127	69.3	175
F	Pretoria, South Africa	Poptart	13	202.8	311
	Santa Cruz, CA	Hamulus	12	219.4	183
	Pittsburgh, PA	Velveteen	14	211.3	311
	Philadelphia, PA	Geralt	14	340.5	144
	Durban, South Africa	Seagreen	14	215.0	311

B.



C.



- **Is tail length is conserved within the bacteriophage clusters and if so, can we use this to potentially predict cluster identity of micrographed phages to help select interesting candidates to send for sequencing?**
- It has previously been shown that the length of the tape measure gene corresponds with the length of the tail in bacteriophages.[2]
- 19 bacteriophages (6 from Drexel, 14 from Phages DB) from clusters B, C and F indicate a correlation between tail length and cluster within 18 of our 19 phages.

Figure 4: Utilization of *tape measure* gene and electron micrograph information to predict mycobacteriophage cluster.

A) Table of several cluster B, C, and F mycobacteriophage. Thirteen separate bacteriophage were selected from Phages DB to compare to the six sequenced bacteriophage isolated at Drexel University. All phages were of clusters B, C, and F to provide adequate comparison between Drexel-isolated phages and previously characterized phages. Table indicates origin of phage, name, the annotated number of the *tape measure* gene, amino acid length of gene product, and the tail length as calculated using calibrated files in ImageJ. B) Comparison of tape measure amino acid sequence (AA) to tail length (nm). Length of *tape measure* gene product was plotted against the bacteriophage tail length. Cluster B tape measure protein lengths ranging from 1900 to 2000 AA. Cluster C tape measure proteins ranged from 145 to 300 AA. Cluster C phages have a myoviridae morphology and, therefore, a contractile tail. Cluster F tape measure proteins ranged from 145 to 311 AA. The phage Geralt was however an outlier in this respect. Despite its relation to Cluster F, its tail length was much longer than the other F phages. C) Using the table in A, we were able to translate our comparative genotypic and phenotypic into Circos, an online tool which produces pham circles. Pham circles compare quantitative data directly and in terms of percentage similarity.

Future Work

- Lysogeny should be verified via incubation of the lawn in 42°C to induce the prophage to initiate the lytic cycle.
- While ion dependence is a relatively straight forward approach to characterize individual phage, more consistent approaches to data collection and analysis would help to compare results between student projects.
- The phage *tape measure* gene and tail length can be used to predict phage cluster, this system may be applied in the classroom in the following ways:
  1. to select different phage clusters when submit samples for sequencing.
  2. to predict phage clusters for phages which are not going to be sequenced so student can identify phages for comparison in their independent projects.

Acknowledgements and References

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1. Cvirkaitė-Krupovič V, Krupovič M, Daugelavičiusa R, Bamforda DH (2010) Calcium ion-dependent entry of the membrane-containing bacteriophage PM2 into its Pseudoalteromonas host. *Virology*, 405, 120-128.
2. Katsura I, Hendrix RW (1984) Length determination in bacteriophage lambda tails. *Cell*, 39, 691–698.