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 mc² 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 body shape, head size, and tail length. 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 phylodynamic relationship of different clusters of phages. Two Drexel students, Christian Schill and Shraya Singh, created a phylogenetic tree based on the tail length of several different phages and found that the proportion of tape measure genome 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 their ability to form lysogens, two phages, Geralt and PhrodoBaggins, isolated by Viktor Evtimov and Shraya Koduri respectively were also sequenced. The temperate phage Geralt readily integrated within M. smegmatis, but PhrodoBaggins was a purely lytic phage. This finding was in agreement with the phage genomes as only Geralt has an integrase gene.

**Bacterial infection cycle.** Bacteria have two different life cycle phases. The lytic cycle mediates the production of new phage viruses by exploiting 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. Mycobacterium smegmatis, B. Phages infect bacteria. C. Phages integrate into host chromosome. Integrate into chromosome by a site-specific recombination mediated by integrase. D. Phages can be 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 prophage genome.

**Drexel's first SEA-PHAGES cohort identified 47 different bacteriophage.** B. Sequenced and 5 were annotated as part of the in silico portion of the course. C. In the Spring term, students were allowed to design and implement their own experiments. This poster reflects the work of several independent projects.

**Genomic and Biological Characterization of Mycobacteriophage Geralt and PhrodoBaggins.**

**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 mc² 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 body shape, head size and tail length. 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 phylodynamic relationship of different clusters of phages. Two Drexel students, Christian Schill and Shraya Singh, created a phylogenetic tree based on the tail length of several different phages and found that the proportion of tape measure genome 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 their ability to form lysogens, two phages, Geralt and PhrodoBaggins, isolated by Viktor Evtimov and Shraya Koduri respectively were also sequenced. The temperate phage Geralt readily integrated within M. smegmatis, but PhrodoBaggins was a purely 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 mrsa regions of Geralt plaques, Geralt-infected M. smegmatis 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 lyase. 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, the lysogen could not be infected by Superphikiman. 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 379.4 nm long and capped at 76.7 nm in diameter. Scale bar 300 nm. B. Geralt Lysogen Purification on 7H10. C. Geralt does not live the Geralt-integrated lysogen verifying stable integration. D. Superphikiman, confirmed later to be a Cluster J phage, forms the Geralt-integrated lysogen confirming a lack of relatedness between Geralt and Superphikiman.

**Lytic Bacteriophage PhrodoBaggins: Characterizing infection efficiency**

**Figure 3: Characterization of PhrodoBaggins as a lytic phage.**

- A) Electron micrograph of PhrodoBaggins. The tail is 379.4 nm long and capped at 76.7 nm in diameter. Scale bar 300 nm. B) Geralt Lysogen Purification on 7H10. C) Geralt does not live the Geralt-integrated lysogen verifying stable integration. D) Superphikiman, confirmed later to be a Cluster J phage, forms the Geralt-integrated lysogen confirming a lack of relatedness between Geralt and Superphikiman.

**Future Work**

- Lysogen 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 straightforward 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 are a key characteristic of the bacteriophage 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.

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