## **Isolation and Analysis of Bacteriophages SoilDragon and Rimmer**

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#### Abstract

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The student participants in our second cycle of the SEA-PHAGE project were again members of the interdisciplinary program Introduction to Natural Sciences, a year-long, full-time learning community with integrated instruction in biology and chemistry. During the fall quarter students collected and purified phages using enrichment cultures of Mycobacterium smegmatis mc2 155 as a host. This year 33 phages were isolated from local soils, purified, and entered into the PhagesDB collection. These will be archived. This set of phages had their DNA purified and analyzed by restriction enzyme digestion and gel electrophoresis. Successful DNA extractions were completed for 30 of the phages. There were 28 successful restriction enzyme experiments that revealed in some cases extremely different cutting patterns. Phages were also analyzed by transmission electron microscopy after negative staining with uranyl acetate. This resulted in clear images of 30 of the isolated phages, all of which appeared to be siphoviridae.

#### **Transmission Electron Microscopy Imaging**

Samples of high titer lysate were prepared and sent to Washington State University for TEM imaging. Images (shown right) revealed siphoviridae morphology. Rimmer's capsid measured approximately 65nm in diameter, tail 275nm in length, for an overall measured length of 340nm. Soil Dragon's capsid measured approximately 65nm in diameter and tail 200nm in length, for a total measured length of 265nm. Additionally, Many of SoilDragon's capsids appeared to be broken in the TEM images. Upon DNA analysis students discovered that the genes identified as coding for the formation of connective tissue were much shorter in SoilDragon than they were in closely related phage (e.g., Jibu08).

# **Conclusion/Future Reasearch:**

Using the SEA-PHAGE wet lab protocols, DNAMaster gene annotation software, and Phamerator and dot-plot comparisons the two phages were examined in detail. HHPred was also used to assist in determining possible protein functions. By looking at the comparisons of these phage genomes to their most homologous counterparts, the distribution, and conservative nature of certain genetic features became more apparent. In both comparisons, it appeared that extensive regions of near genetic identity and functional synteny were broken by discrete areas of high divergence. This fits the well-known pattern of the mosaic architecture of phage genomes. In particular, the number of phams that are unique to the Cluster E phages make a detailed examination of their potential functions in Rimmer an interesting challenge.

Analysis of genes and their features with the software used such as the start and stop codons, Shine-Dalgarno sequences, coding potential, gaps and overlaps, and looking at protein comparisons helped to establish a stronger familiarity with the terminology and a better comprehension of genomics in general. Future research directions include attempts to isolate lysogens of these phages, determination of immunity groups, and the parameters of their life cycles such as burst size. Pedulla et al. (2003) Origins of Highly Mosaic Mycobacteriophage Genomes; Cell 113: 171-

### **Plaque Morphology**

When released onto a lawn of Mycobacterium Smegmatis, SoilDragon produced moderately clear plaques of two distinct sizes (approximately 2.5mm) and 5 mm.) Rimmer produced clear plaques of three distinct sizes: Small (less than 5mm in diameter), Medium (approximately .75mm in diameter), and Large (1.5mm in diameter). The variability of plaque sizes produced by a singular isolated phage was confirmed through consistent results yielded from repeated testing.



Isolated DNA samples of each phage were treated with the following restriction enzymes; BAMH1, H1nDIII, Clal. EcoR1, HaellI, and then loaded onto agarose gel for separation analysis by electrophoresis. Image Results (shown right) were then compared to the enzyme digest patterns of other known phages. Although Rimmer and SoilDragon produced similar enzyme restriction results and a GC difference of only 1% analysis of their DNA sequence revealed that they were genetically diverse and distinct.





#### Rimmer







SoilDragon was named for its ability to destroy bacteria and because it was extracted from the soil SoilDragon was harvested from a molehill near Black Lake in Western Washington just a few miles away from The Evergreen State College Lab where they were isolated, purified, and grown using HHM SEA-PHAGES protocol.

**Origin and Naming** 

#### SoilDragon







These incredibly similar genomes had very little variation. Gene #5 in Jobu08 was 390 Bp longer then that of SoilDragon. This gene expresses collagen triple bond helix repeat, for formation of connective tissue. It was also observed on Jobu08, on the Gene#25 coding for Tail essembly chaperone, there is a **Translational frame shift.** 

There is a group of clusters present in both phages that all have 54 in the Family suggesting that all 54 share the cluster of E specific proteins, this is seen throughout the entire plot. There is a large gap at Gene #37, this insertion/deletion has an additional 6 genes on **Rimmer only, all of them of unknown function** and not cluster E specific. Gene #132 only Williez in family, and Gene # 146 only Rimmer in the family. One that was found that wasnt exentuated on phamerator Gene #101 was only **`on Williez and is Punitively DNA binding motif** found in homing endonuclease.

Rimmer was adopted on October 31st, 2014. Like many phages, Rimmer has a large head (i.e., capsid). Seeing as you are what you eat, and these phages "feed" on M.Smeg, they must be a "Smeg Heads". Therefore these phage have been named for the character Arnold Rimmer, a.k.a "Smeg Head", from the British Space Opera Spoof Red Dwarf.

INS group 40

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#### **SoilDragon vs. Rimmer:** Although these were both Siphiviridae phages, had similar plaque morphologies, and similar results from the gel electrophoresis, they not only were grouped into two separate clusters, A3 and E, but they shared no genomic similarities when processed by gepard or Phamerator.

#### SoilDragon vs. Jobu08:

#### **Rimmer vs. Williez:**