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Isolation and Characterization of an A4 Mycobacteriophage from Central Illinois

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INTRODUCTION

Bacteriophages are easy to use in research because of their easily culturable make-up; this makes bacteriophages, viruses that infect and replicate inside bacteria, an ideal model organism to study for a variety of purposes including food products, counteracting biotoxins, and studying principles of ecology and evolution. Illinois Wesleyan University's current students of 2014-2015 General Biology class took part in the Howard Hughes Medical Institute SEA-PHAGES program to research bacteriophage population diversity. In the fall semester, students collected samples from various environments and then isolated their individual phage in the lab through a soil enrichment technique. The phages were then purified and characterized. Finally, the phage Morrow was chosen to be sequenced.This spring semester, students analyzed Morrow's genome through annotation and bioinformatics using programs such as DNA Master and Phamerator. Through this, genes with protein sequences can be identified. These results are significant in contributing to the understanding of mycobacteriophage diversity.

MATERIALS AND METHODS

1.Phage Isolation and Characterization

Each student collected a soil sample from the Midwest region and utilized *Mycobacterium smegmatis* mc^2155 cultures as the host bacteria for phage infection. Morrow was collected from just outside the Morrow Plots at the University of Illinois at Urbana-Champaign shown in Figure 1. Using provided procedures from the manual published by Science Education Alliance such as direct plating or soil enrichment, phages were isolated and identified. Once a pure population of a single phage was isolated, its plaques were characterized and its DNA was isolated and analyzed using enzyme digests.

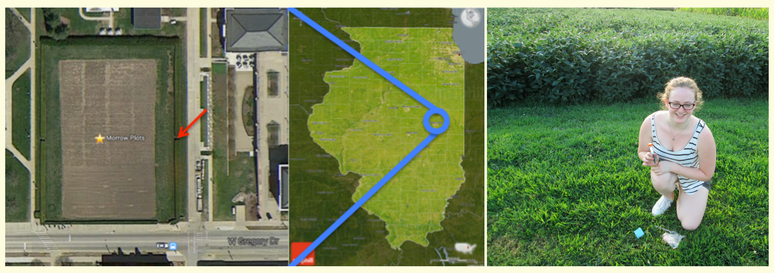

Figure 1: Collection site

2. Genomic Characteristics of Morrow

Morrow's DNA was isolated and complete sequencing was performed at the Pittsburgh Bacteriophage Institute. The genome was sequenced using Illumina sequencing where the approximate shotgun coverage was 2696. The genome was annotated for ORFS, tRNA, tmRNA's and other various features. DNA Master, Phamerator, HHpred, and BLAST were computer programs used for genomic analysis.

RESULTS AND DISCUSSION

1.Isolation and Plaque Characterization

Morrow was collected just outside of the Morrow Plots at Illinois University-Urbana Champaign 2.5cm into the soil. The soil was slightly damp and dark colored. Morrow was isolated using the soil enrichment protocol.

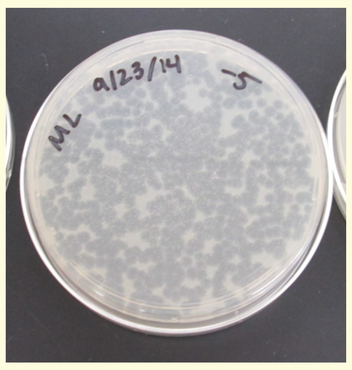

Figure 2: Plaque morphology

Morrow's DNA was isolated through the enrichment culture technique. This protocol creates conditions that favor replication of specific bacterial phages. This isolation method is phage specific to Mycobacterium smegmatis.

The phage produced a single phenotype. Morrow has the siphoviridae morphotype. The plaque morphology is approximately 3-4mm in diameter and are clear as shown in Figure 2. The clear plaques indicate that Morrow is a lytic phage. Lytic phages replicate and reproduce inside the cell, causing cell lysis as it releases new phages. Morrow's clear plaques show most of the bacteria lysed as a result of the phage.

2. Restriction Digest

Restriction digest patterns help determine how each phage is unique. Restriction enzymes were mixed with Morrow's DNA sample to cut restriction sites. Then a gel electrophoresis was performed to separate the cut fragments.

Only the sample mixed with the HaeIII enzyme was cut while all others looked identical to the uncut sample. The cause could be that Morrow's DNA only contained HaeIII restriction sites, or the enzymes used were defective. As this restriction pattern is seen in other A4 phages, it is likely Morrow only contains HaeIII restriction sites. The restriction digests for Morrow and BellusTerra, a closely related phage, are shown for comparison, in Figure 3 and Figure 4, respectively.

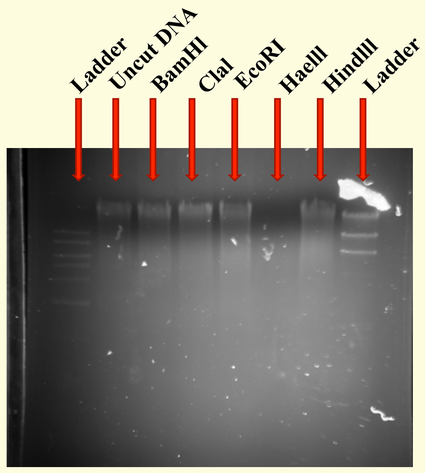

Figure 3: Restriction Digest of Morrow

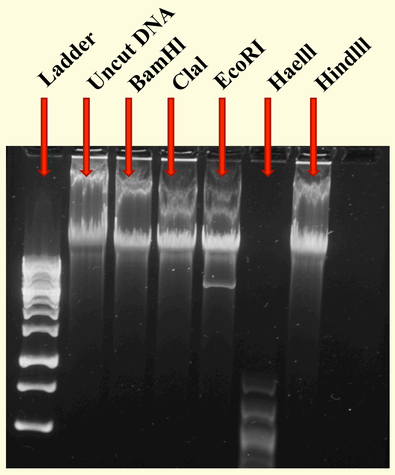

Figure 4: Restriction Digest of BellusTerra

3.Immunity Testing

Immunity testing helps determine relationships between the newly discovered phages. Bacterial colonies that were immune to Morrow were selected for and tested for immunity to seven other phages in the class. Three colonies produced enough bacterial growth to be plated and spotted with other phages. The results are shown in Table 1. From these results, it can be inferred Morrow is related to MickyD, Colesidwell96, and possibly Audrizzle82 and Benedict11, because the bacteria selected for its immunity to Morrow, was also at least partially immune to these phages.

These results may be considered abnormal and indecisive, as the bacteria selected for its immunity to Morrow was only partially immune.

Table 1: Immunity Testing Results for Morrow

Phage	Resistance		
	A	B	C
Morrow	Partial	Partial	Partial
MickeyD	Total	Total	Total
Colesidwell96	Total	Total	Total
Ovington32	None	None	None
Minky	None	None	None
Audrizzle82	Partial	None	Partial
Pavarotti	None	None	None
Benedict11	Partial	Total	None

4.Isolation of DNA

From the phage head made up of virtually all protein, Morrow's DNA was isolated and purified. The phage was incubated with enzymes called nucleases DNase I and RNase A to disrupt the structure of nucleic acids. The buffer resin was then added after the phage precipitated in order to denature the coat proteins and enzymes which allows genomic DNA to bind to the resin. The DNA was washed with isopropanol to remove the excess denatured proteins and salts. Finally the phage genomic DNA was eluted with a hot buffer and was ready for analysis through gel electrophoresis shown in Figure 5.

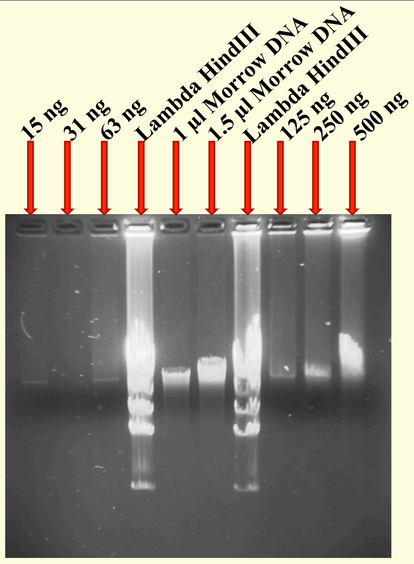

Figure 5: Gel Electrophoresis of Morrow's DNA

5.Comparison to the Average A4 genome

Morrow is categorized in the A4 subcluster and has many characteristics similar to its cluster, as seen in Table 2. Morrow's base pairs in comparison to the average A4 is slightly larger. Morrow's larger gene size is consistent with the higher number of base pairs. Morrow lacks tRNA, which is common among A4 genomes. Morrow and the average A4 have a similar Guanine/ Cytosine percentage content only differing by 0.1%

The lack of differences between the A4 subcluster and Morrow suggests that Morrow is consistent with other bacteriophages in the A4 subcluster, as shown in the next section.

Table 2: Comparison between Morrow and other A4 Phages

	Size (bp)	GC%	Genes	tRNAs
A4	51,395	63.9	86.5	0
Morrow	51,411	63.8	91	0

6.Comparisons to a Closely Related Completed Phage

Morrow and BellusTerra have similar characteristics within their genomes, as seen in Table 3. Morrow's base pairs in comparison to BellusTerra's is slighter larger. Morrow's larger gene size is consistent with the higher number of base pairs. Both Morrow and BellusTerra lack tRNA which is common among the A4 genomes. Morrow and BellusTerra have a similar Guanine/ Cytosine percentage content only differing by 0.1%. The high E value of BellusTerra, 7.746e+04, shows the relatedness of the two genomes in relation to length and subcluster.The extensive similarities between Morrow and BellusTerra suggests that both are consistent A4 bacteriophages, and verifies their relatedness.

However, the genomes are not entirely identical and are different in some areas as shown in Figure 7. The genomes do not greatly differ until very late in the genomes, where Morrow's extra 200 base pairs are. Figure 6 shows an electron microscopy of BellusTerra.

Table 3: Comparison between Morrow and BellusTerra

	Size (bp)	GC%	Genes	tRNAs
BellusTerra	51,236	63.9	89	0
Morrow	51,411	63.8	91	0

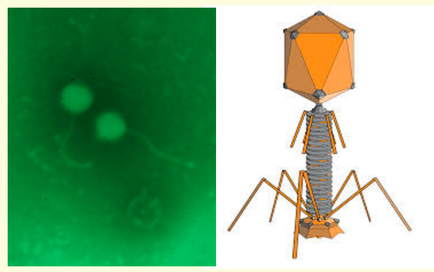

Figure 6: Electron Microscopy of BellusTerra Compared to the Typical Bacteriophage Structure

CONCLUSIONS

Bacteriophages are the most numerous viruses on the planet. Through the SEA-PHAGES program, students were able to study their own bacteriophage. From the initial phage isolation to the genomic analysis of Morrow, the SEA lab students have learned to utilize many skills and techniques over the course of the school year. The wet lab portion focused on techniques used in microbiology, molecular biology, and electron microscopy to characterize the environmentally isolated phage Morrow. The genomics experience focused on using bioinformatics tools to facilitate genome annotation. This allows for the comparison to other bacteriophages and gains insight into the diversity of mycobateriophages in the environment. This information helps provide distinction among other phages and allows for the analysis of what exactly makes Morrow unique and different. By exploring the world of mycobacteriophages, other researchers and scientists can utilize this information and delve into a range of environmental, health, biomedical, and ecological applications.

References/Acknowledgments

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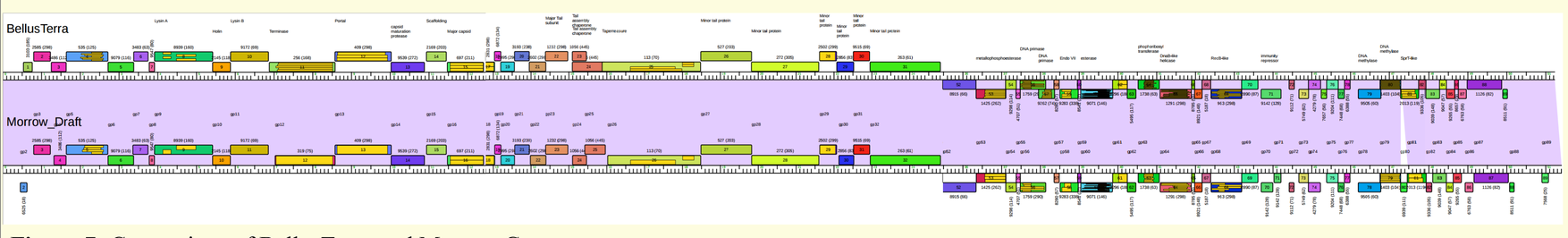

Figure 7: Comparison of BellusTerra and Morrow Genome

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