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Future in Phage Therapy Against Antibiotic-Resistant Bacteria

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University of
Mary Washington

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with help from the 2021-2022 Phage Hunters Class

Background and Objective

Background: The genus *Bacillus* is a medically important genus because several species cause disease like *cereus* and *anthracis*, that involves several species within it. We chose *B. thuringiensis*, a non pathogenic species of Bacillus, as our host as it is very abundant in the environment and therefore it is easy to isolate phages. The University of Mary Washington Class of 2021-2022 isolated a total of eight phages. The phages were photographed using a Transmission Electron Microscope to determine their morphotype. They were further characterized by host range analysis showed that KoopaTroopa grew on other Bacillus species such as *B. cereus*. *B. cereus* is a foodborne pathogen that can cause gastrointestinal illness in humans. Since the goal of phage research is to develop phage therapy against pathogenic bacteria, we analyzed the genome of KoopaTroopa further. Through genome annotation we hope to find lytic and toxin producing genes that help Koopa Troopa destroy the pathogenic *Bacillus* species.

Objective: To isolate and characterize new phages using *Bacillus Thuringiensis* subsp. *Kurstaki* as a host, in order to find unique phage genomes that could be used for future phage therapy for pathogenic *Bacillus* species.

PCR Gel

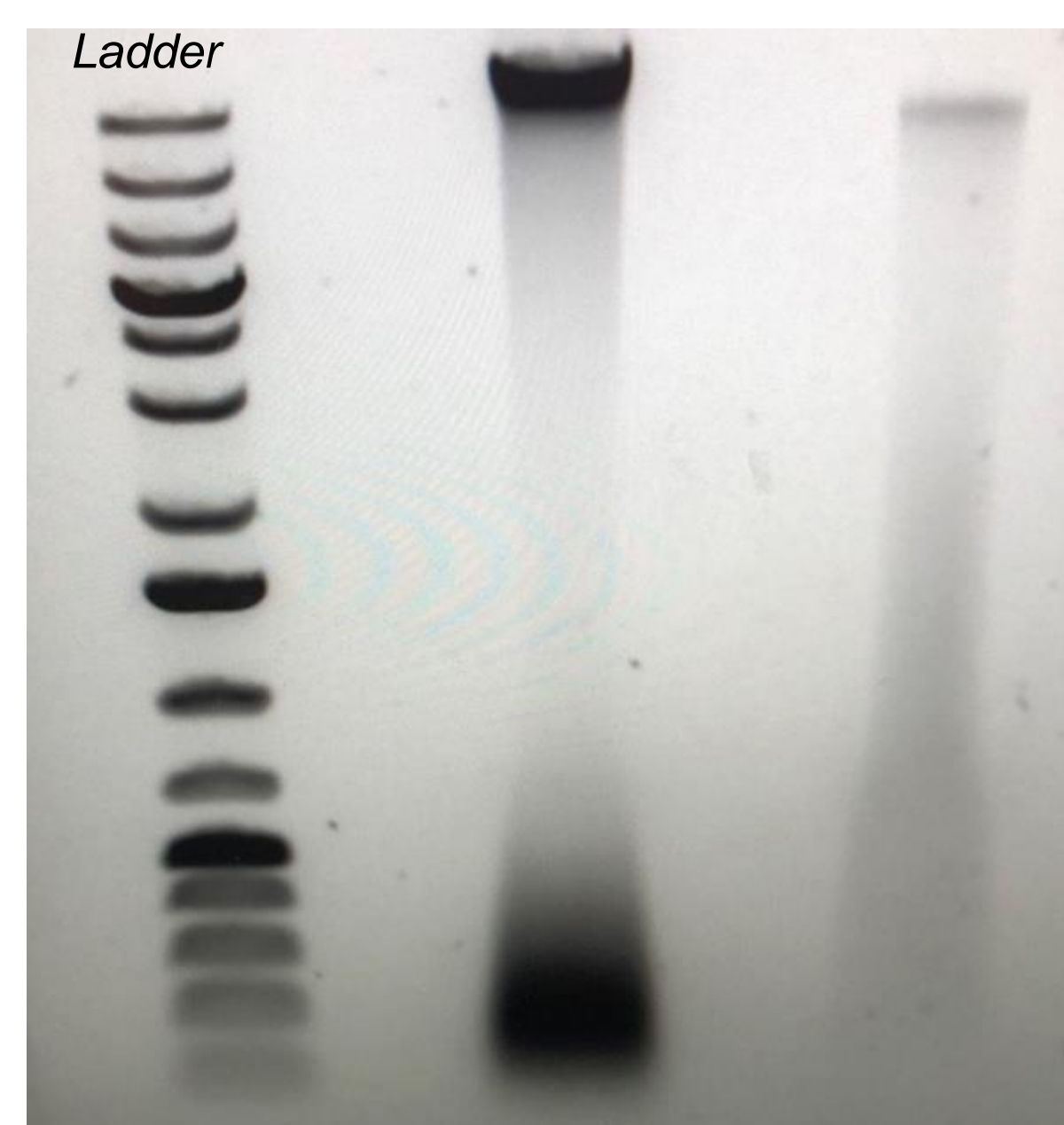


Fig 1, A PCR Gel was run to determine the length of bands extracted from an alternate DNA extraction protocol on two other phages, Jabberwock and ToucanSam.

TEM analysis of Phages

Isolated bacteriophages were imaged with TEM to measure the diameter of the capsid and length of the tail. Koopa Troopa had a longer non-contractile tail length of approximately 136.8 nm making it a Siphoviridae.

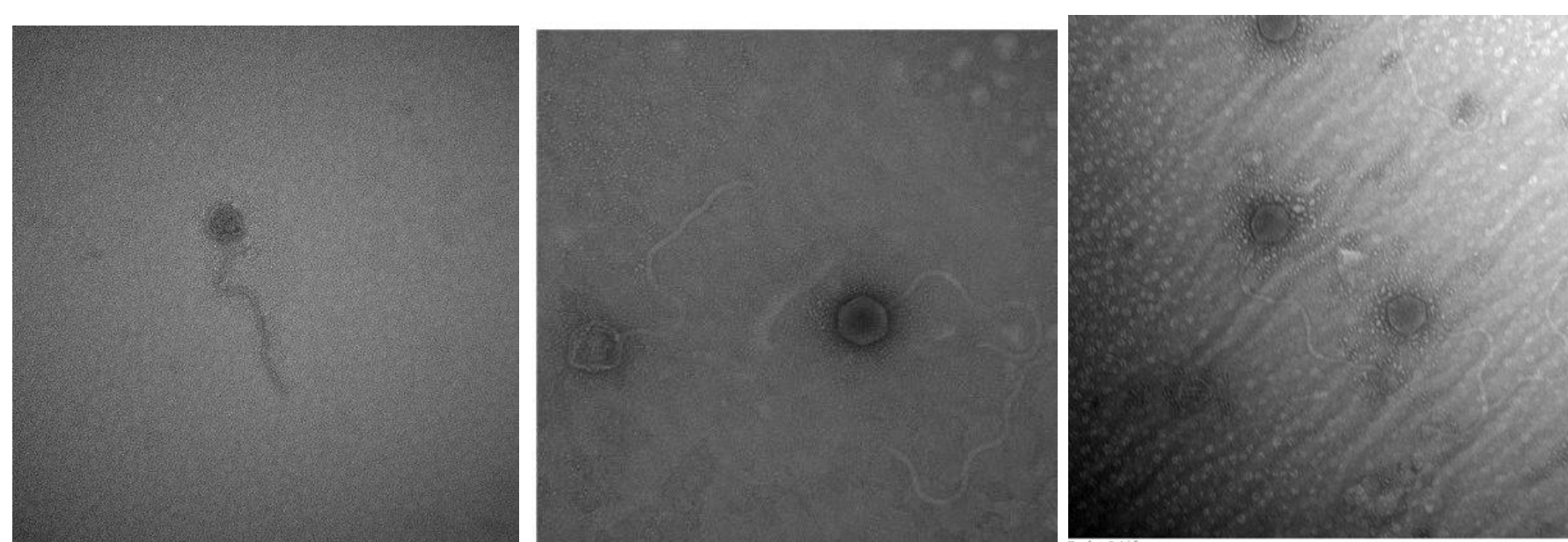
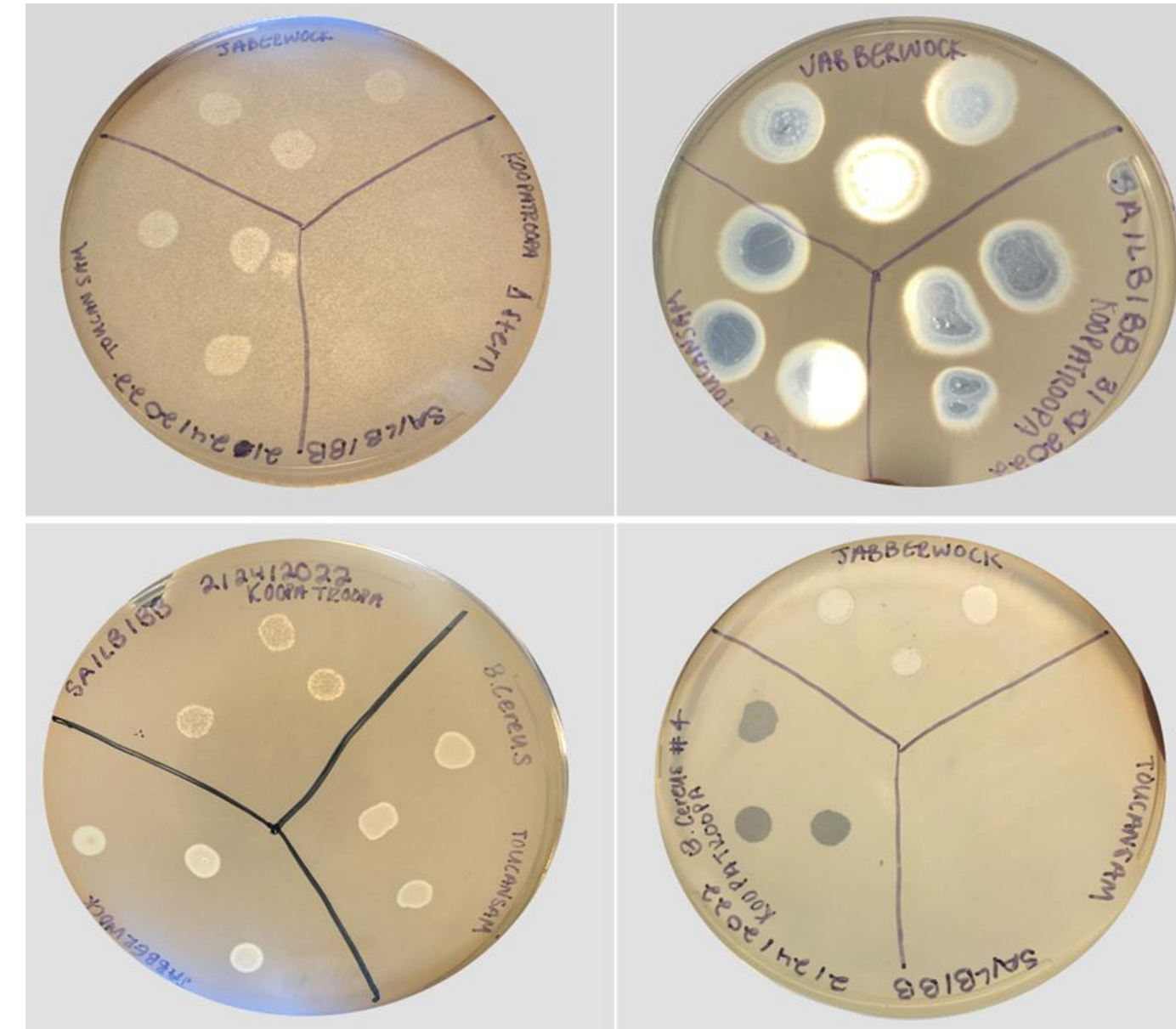


Fig 2-4. TEM images of the eight isolated bacteriophages.

Host Range analysis

A host range was performed by adding bacteriophage lysate from each bacteriophage on *B. cereus* #4, *B. cereus* strain 2,, *B. anthracis* delta sterne, and BTK. Both *B. cereus* and the *B. anthracis* delta sterne strains are pathogenic to humans. This was done to see how the isolated bacteriophages respond to various strains of *Bacillus*.



	Bacillus Anthracis Delta Sterne	Bacillus Cereus OG 14579 (#4)	BTK
Koopa Troopa	-	+	+
Jabberwock	+	+	+
Toucan Sam	+	-	+

Fig 5 and 6. Host range analysis of the four isolated bacteriophages. Koopa Troopa lysed several medically important strains of *B. cereus* and *B. anthracis* strains.

Koopa Troopa genome (characteristics at a glance)

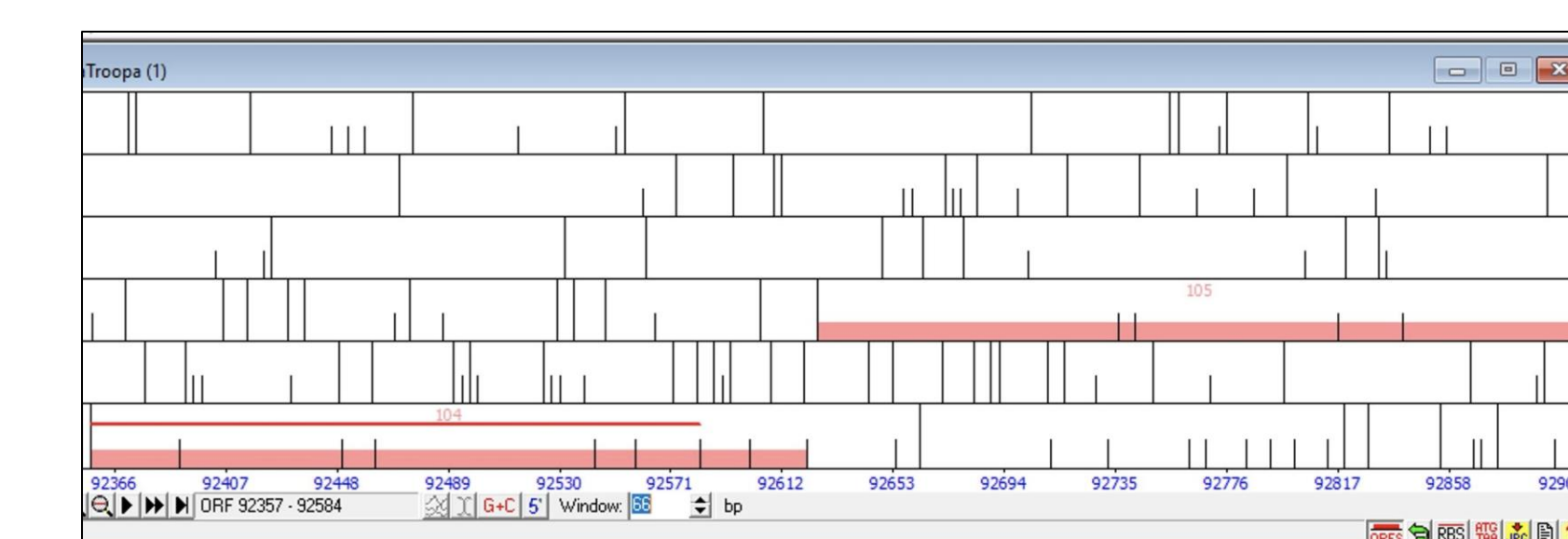
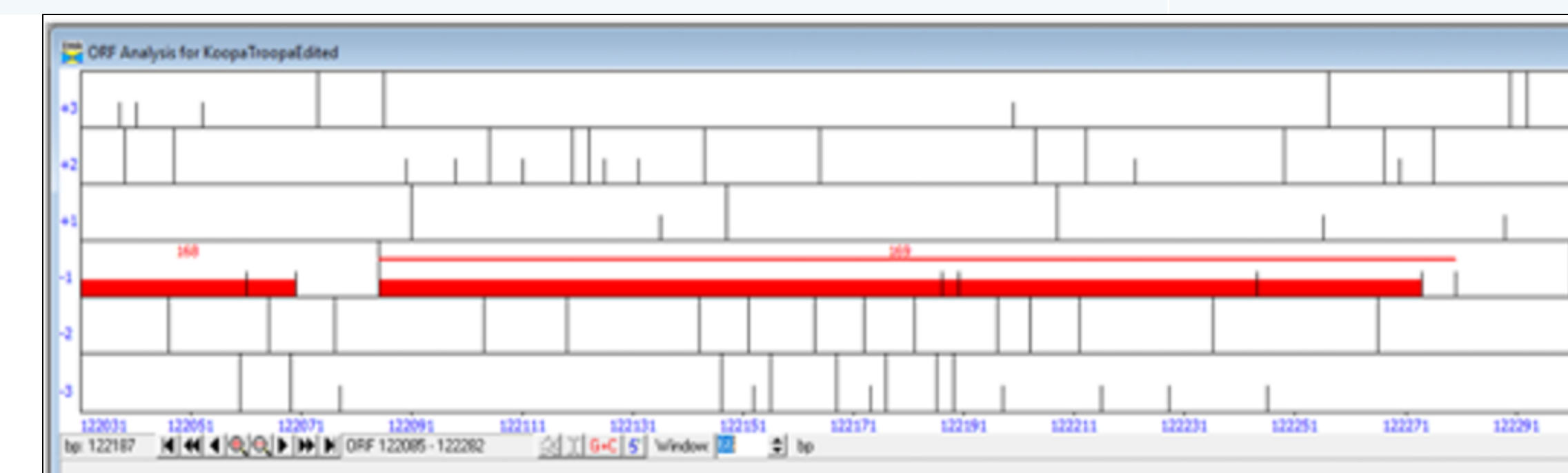
Many genes in the KoopaTroopa genome have similarities to a previously discovered phage, Tsamsa. Tsamsa was isolated from a sample that was collected in Africa from a Zebra that died from *anthrax*. It was also shown to lyse several other *Bacillus* species including medically important ones like *B. cereus* several *B. thuringiensis* strains, and several *B. anthracis* strains. Tsamsa like Koopa Troopa is also a Siphoviridae with a tail length of 440nm (Klump et.al., 2014 PLOS One). This makes Koopa Troopa really interesting and important to characterize

- **Length of genome** 170590 bp including 284 terminal repeat
- **Top 3 BLAST matches** Izhevsk, Skywalker, Chewbecca
- **Autoannotated features**
 - 64 genes in the forward direction
 - 205 genes in reverse direction
 - 20 genes from tRNA

Positional genome annotations

Some examples of manual genome annotations made based on BLAST matches, coding potential, and RBS scores:

Annotated genes	Changes made to the start site
Replication protein (Gene 22)	Extended by 6 bp
Terminase, large subunit (Gene 35)	Shortened by 6 bp
Gene 136	Extended by 7 bp
Gene 169*	Extended by 226 bp
Signal peptide (Gene 104)	Extended by 39 bp
Trimeric dUTP diphosphotase (Gene 123)	Shortened by 6 bp



Identified functions of genes

- Gene 15 – lysin
- Gene 34 – terminase small unit
- Gene 35 – terminase large unit
- Gene 36 – capsid maturation protease
- Gene 43 – major capsid protein
- Gene 45 – tail assembly chaperone
- Genes 46, 48, 51, 53, 55, 56 – tail proteins or tape measure proteins
- Gene 61 – holin

Unusual features of the genome

- Gene 22 is reverse in between forward genes
- Gene 90 is forward in between reverse genes

Similarities of Function to Tsamsa

- Gene 96 : DNA ligase, ATP-dependent

Functions not identified

- Major capsid proteins
- HNH endonuclease
- Scaffolding protein

Conclusion & Future Direction

Of the eight phages isolated in the Fall Phage Hunters class, one of which, Koopa Troopa was sent to SEA-PHAGES for sequencing and annotated. Excitingly, several phages grew on pathogenic strains of *Bacillus* showing a potential for phage therapy to combat developing antibiotic resistance in pathogenic *Bacillus* strains. Future research can further annotate the interesting tRNA genes of the Koopa Troopa genome and general characterization.

We would like to thank Dr. Agrawal for her constant contribution to our project and our education, to the 2021-2022 Phage Hunters class for their help and support, to the Department and Dr. Lewis for allowing us to do our research, and finally to the SEA-PHAGES program for an amazing program.