

In Silico Genomic Annotation of Kady, a Novel Mycobacteriophage Isolated from Soil



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Abstract

The purpose of this research project was to isolate, name, sequence, and analyze a newly-discovered mycobacteriophage. A mycobacteriophage is a virus that destroys a bacterial cell by taking over the internal machinery of the host bacterium. In our study, we discovered a novel, lytic, Siphoviridae mycobacteriophage that we named Kady. Kady's known bacterial host is *Mycobacterium smegmatus*, but there could be other bacterial hosts for it as well. Kady was isolated from an enriched soil sample in the fall and was later sent to a sequencing facility to have its DNA analyzed. The sequenced file of Kady's DNA provided us with the data needed to further annotate Kady's genome. We used bioinformatics tools, such as DNA Master, a genome annotation software that predicts a bacteriophage's gene location, as the basis for our gene annotation studies. We also utilized several additional software programs to cross-analyze the genomic data with other sources and with similar species of bacteriophage. GeneMark and Glimmer were useful at determining the coding potential for various start codons along Kady's genome. Phamerator, Starterator, and BLAST analysis tools were efficient at comparing the genome of Kady and closely related bacteriophage to identify similarities and differences between the genomes. These software programs collectively identified eighty-nine potential genes in Kady's genome, though we only annotated forty-six of these genes. After comparing Kady's genome to genetically similar mycobacteriophage, we altered some of the genes within Kady's genome. We changed the start codon, deleted a gene, or predicted a frameshift for several of Kady's genes based on sufficient data from these software programs. However, the function of the 89 identified genes has yet to be determined.

Introduction

- Kady was isolated from soil found in Warren, MI
- Isolation Host: *Mycobacterium smegmatus*, mc²155
 - Plaque type: Lytic
 - Plaque size: 3mm diameter
- Family/Cluster/Sub-Cluster: Siphoviridae A3

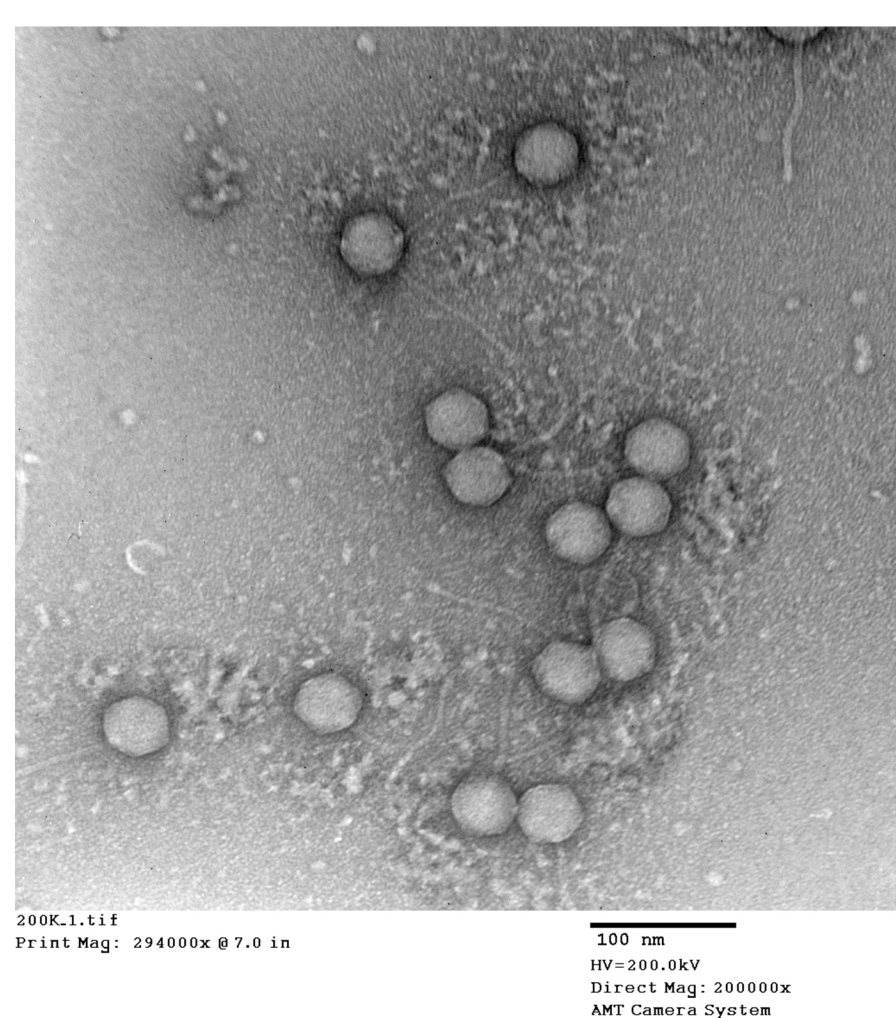


Figure 1. Electron microscope (EM) picture of Kady

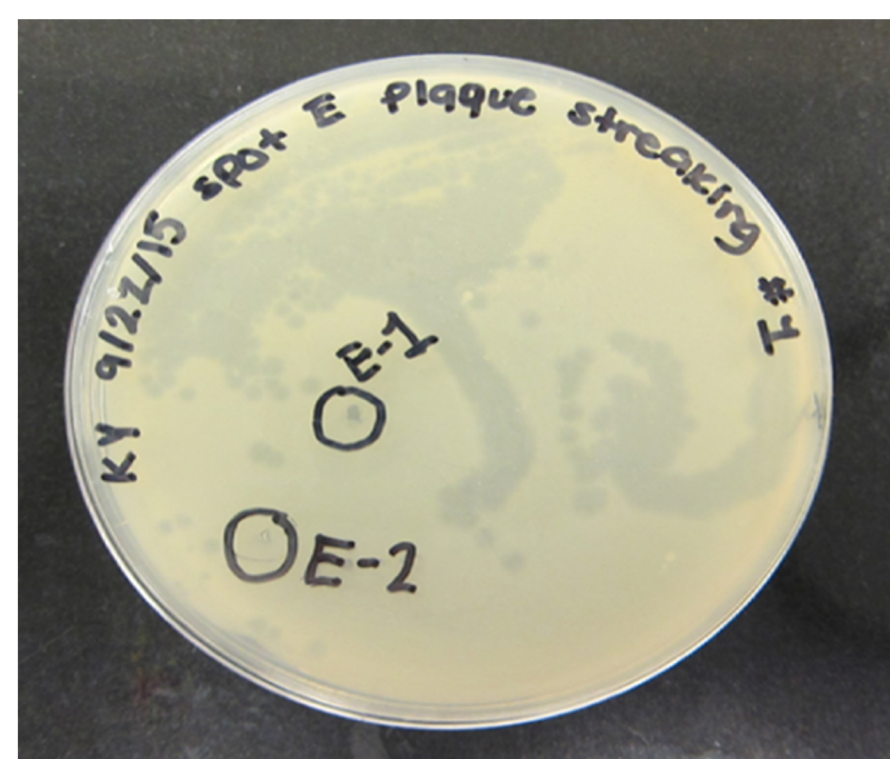


Figure 2. Plaque morphology of Kady

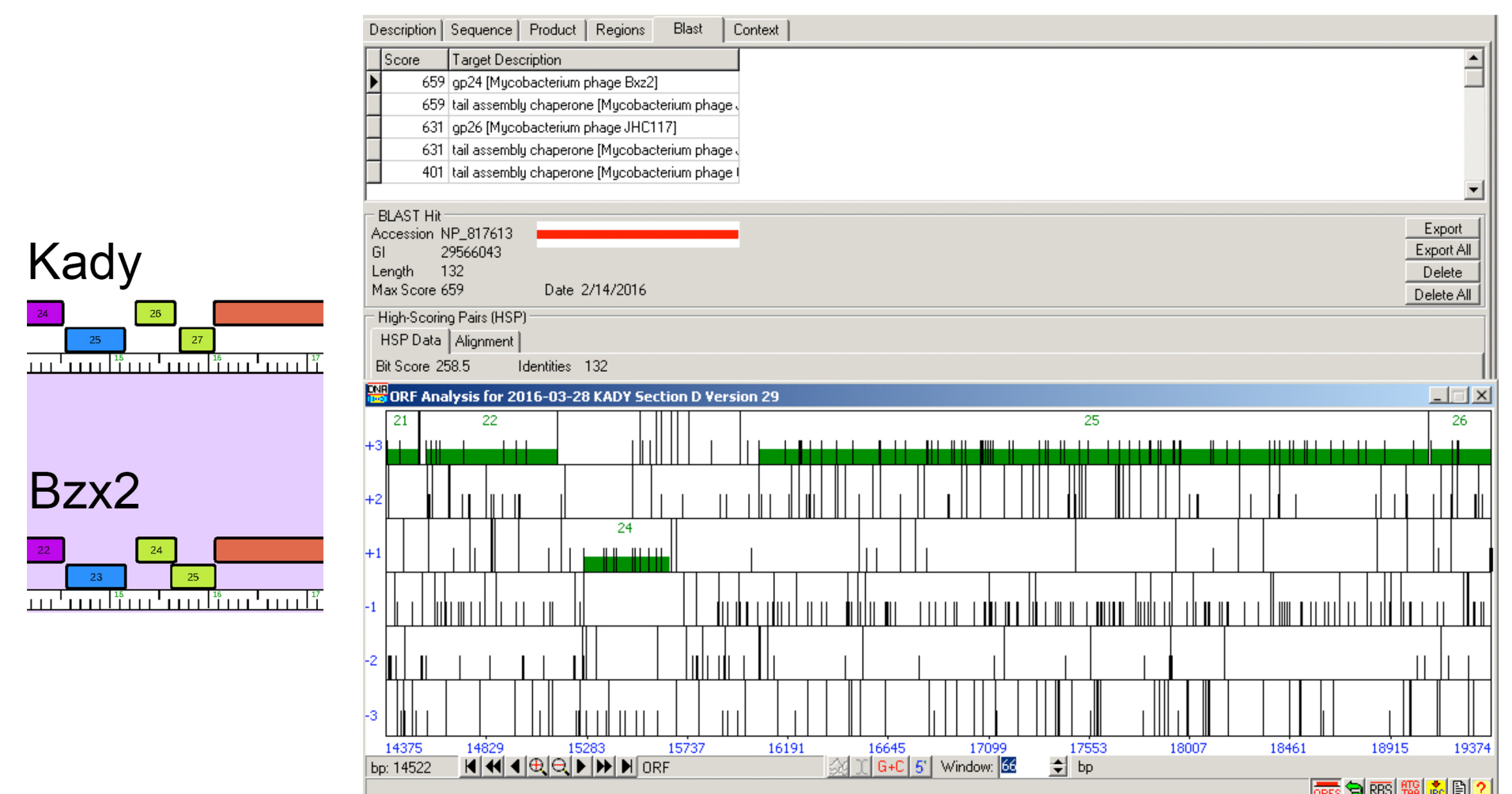


Figure 5. DNA Master and Phamerator Frameshift Location

- Frameshift at genes 23 and 24
- Added a gene between genes 60 and 61
- Changed the start codon of genes 79 and 80
- Deleted gene 89

Conclusion

Further study from the Howard Hughes Medical Institute's Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (HHMI SEA-PHAGES) program could lead to Kady being used for therapeutic uses against other bacteria. Our research will provide us with data to compare other mycobacteriophage to Kady, potentially revealing evolutionary histories and furthering our knowledge of mycobacteriophage in general.

Results

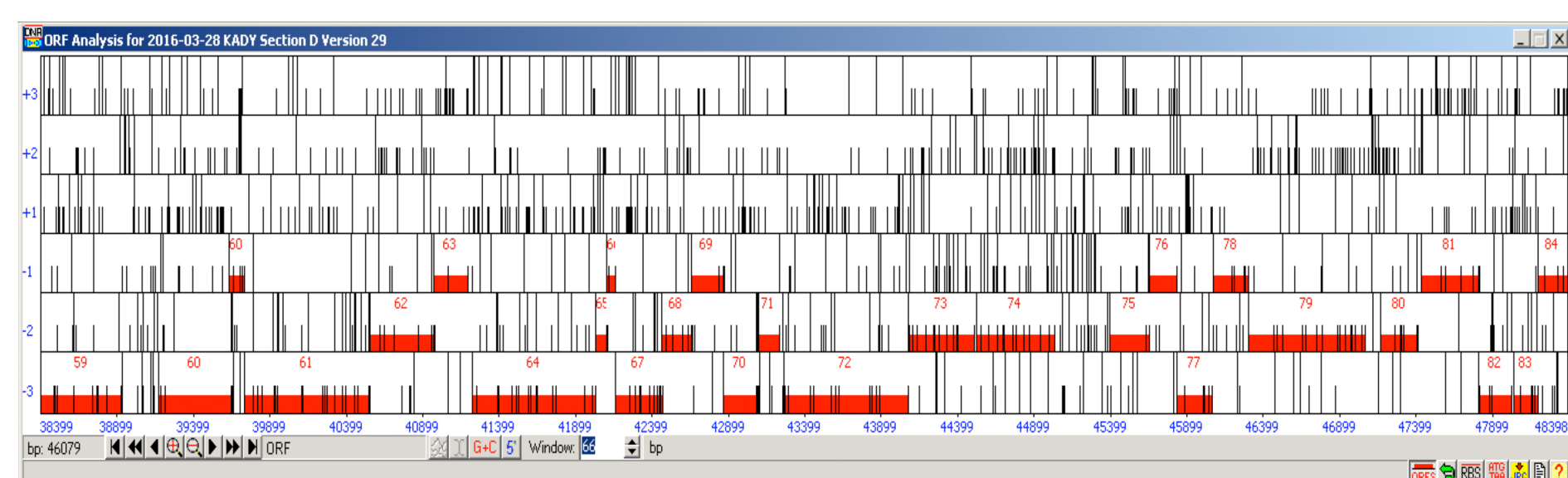


Figure 3. DNA Master reverse gene

- Genome length: 50,898 base pairs
 - GC Content: 64.2%
- Closest Relative: Mycobacteriophage Bzx2
 - Annotated forward genes 17-33
 - Annotated reverse genes 61-89

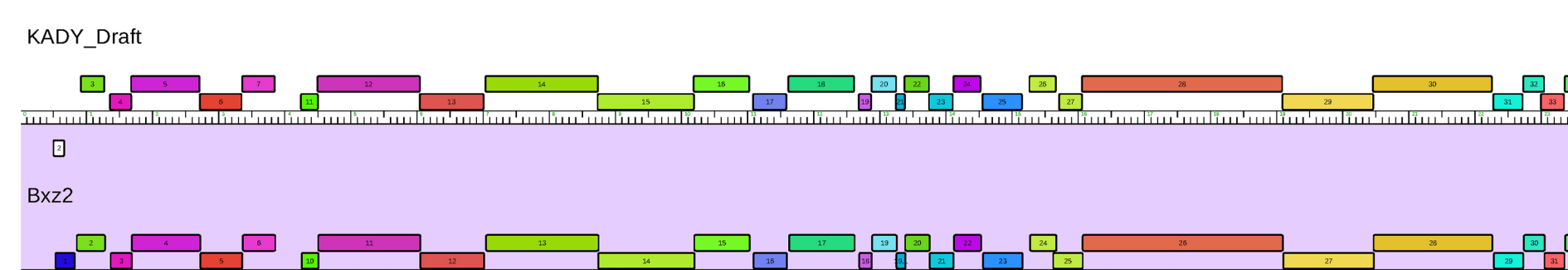


Figure 4. Phamerator map between mycobacteriophage Kady and mycobacteriophage Bzx2

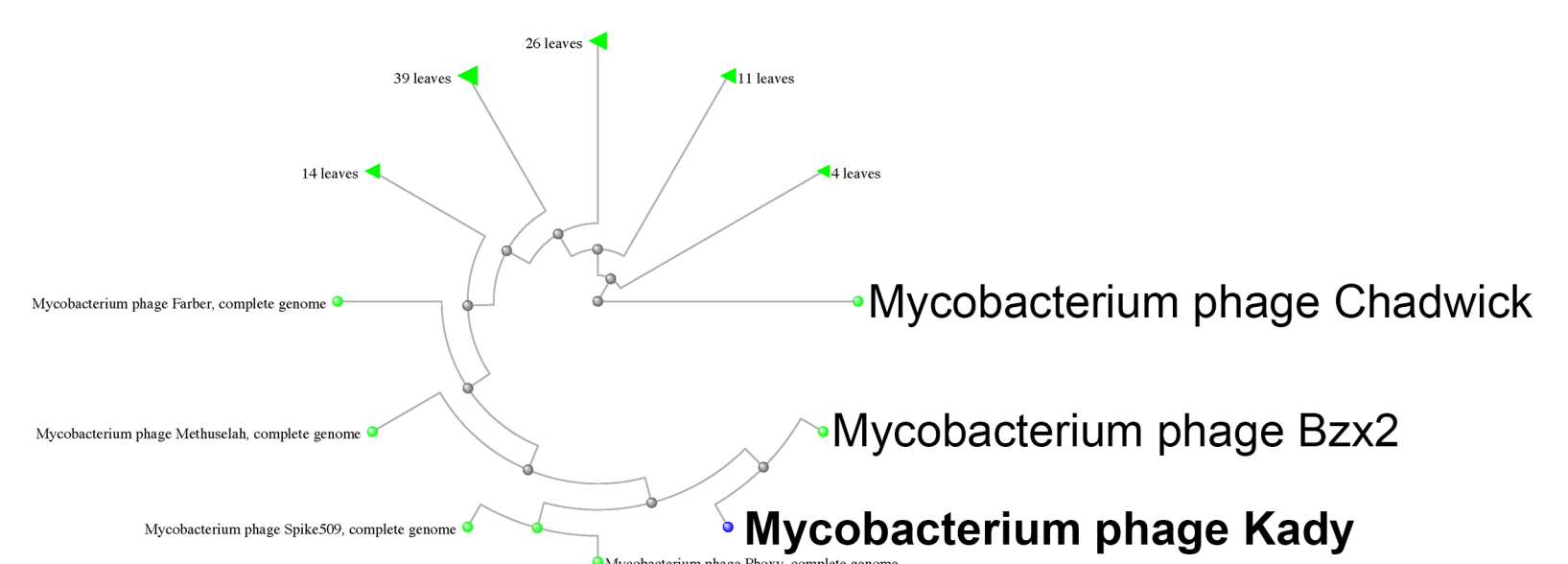


Figure 6. Phylogenetic tree of Kady against other mycobacteriophage

Acknowledgements

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