





Introduction: *What is a bacteriophage?*

A bacteriophage, also known as a phage, is a virus that infects, replicates, and lyses bacteria^{2,7}. Bacteriophages are regularly described as the most abundant and diverse biological entity on Earth. There are more than 10³¹ bacteriophage in the world³.

What is Salmonella and EHEC food poisoning?

Gastroenteritis caused by salmonella and *Escherichia coli* (E. coli) bacteria. E. coli is a human commensal organisms. Most E.coli strains are harmless, but some, such as enterohemorrhagic *E. coli* (EHEC) can cause serious disease. How can Bacteriophage prevent food poisoning -

Reduce bacteria on leafy greens, food processing equipment, etc. with a bacteriophage wash. Collaborators at OSU Stillwater have isolated many anti-salmonella or anti-EHEC bacteriophage. Nothing is known about them.

Purpose:

- Determine which type of phage is J25
- Identify the depolymerase-like gene in J25
- Sequence the J25 depolymerase-like gene

Materials and Methods:

Bacteriophage J25 was obtained from Dr. D. Jaroni at Animal and Food Sciences, OSU Stillwater A Phage J25 DNA sequence was used to identify similar bacteriophage genomes in GenBank. A depolymerase-like gene sequence and flanking regions were identified in the similar genome. PCR primers were designed using this sequence to amplify the gene in whole or part These primers were used to amplify the depolymerase-like gene from J25 by PCR PCR product was purified and sent to the OSU Core facility, Stillwater, OK for DNA sequencing.

Sequence Analysis and Bioinformatics: DNA sequences were analyzed, traces assembled, and primers designed using DNAStar (Lasergene Madison, WI) or SnapGene software (GSL Biotech LLC Chicago, IL). BLAST was used to find similar genomes in GenBank¹. MEGA7 was used to align, compare, and create a phylogeny based on the DNA sequence⁴.

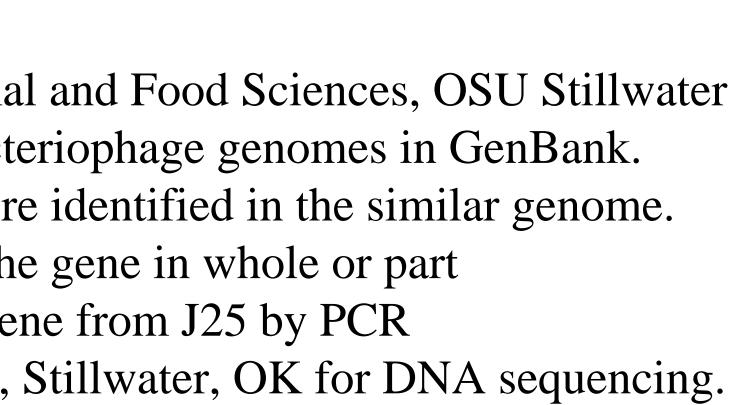
Results:

Summarized DNA BLAST Results:

Query Coverage	Identity	Bacteriophage
100%	99.64%	Shigella phage Sfl DNA
100%	99.51%	Escherichia phage vB_EcoM_G2133
100%	99.39%	Escherichia phage DNA

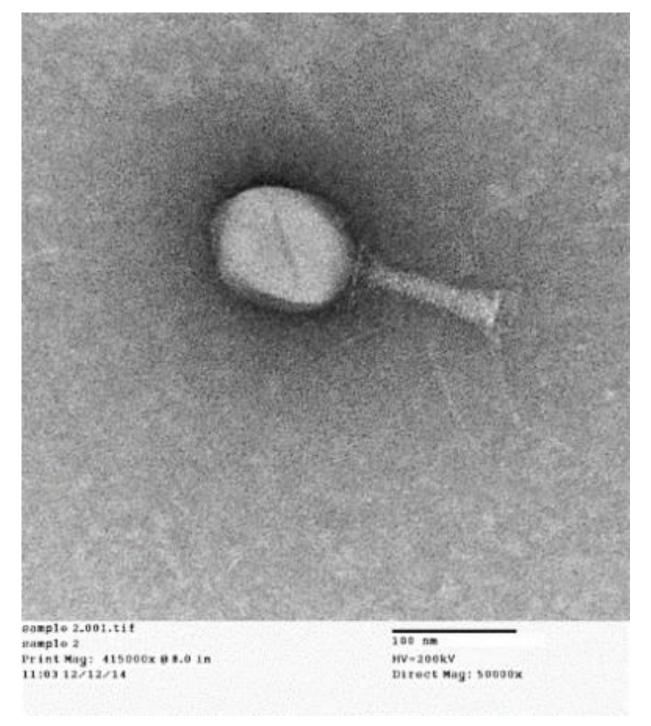
Cloning and Sequencing of the Depolymerase-like Gene from Bacteriophage J25

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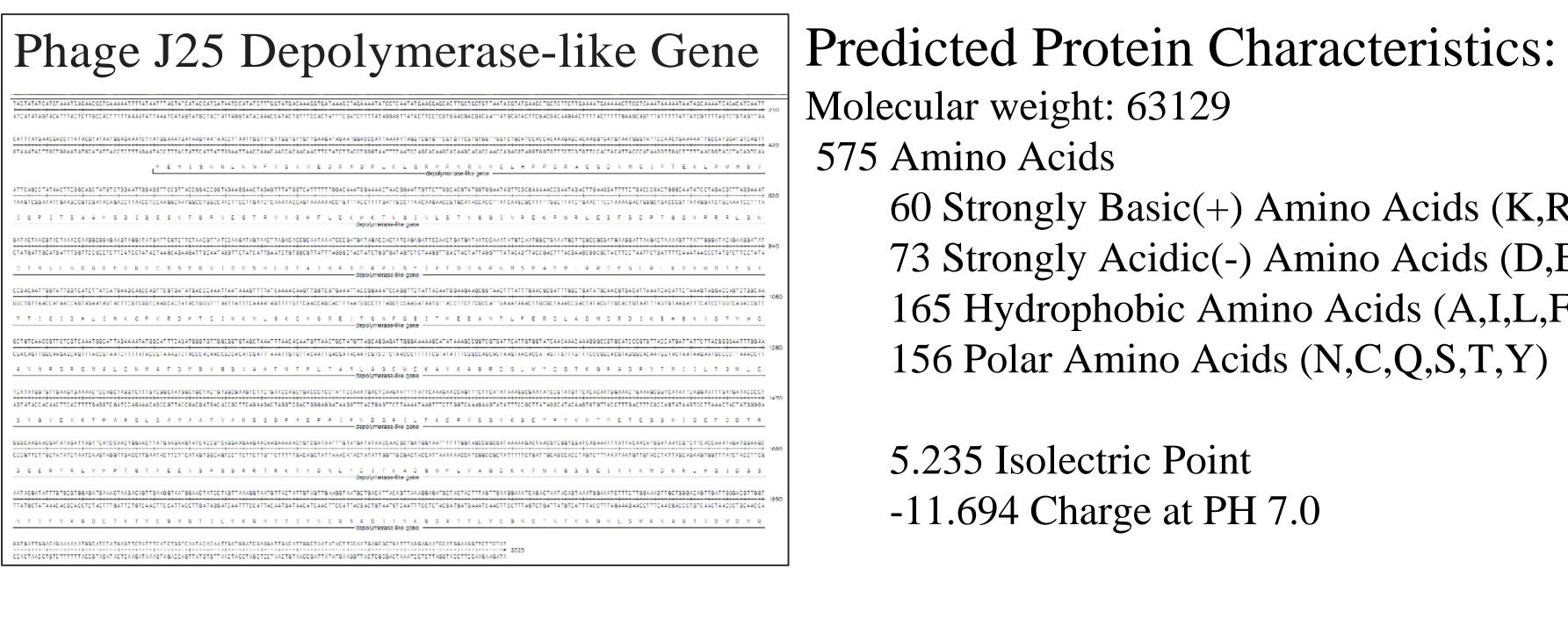


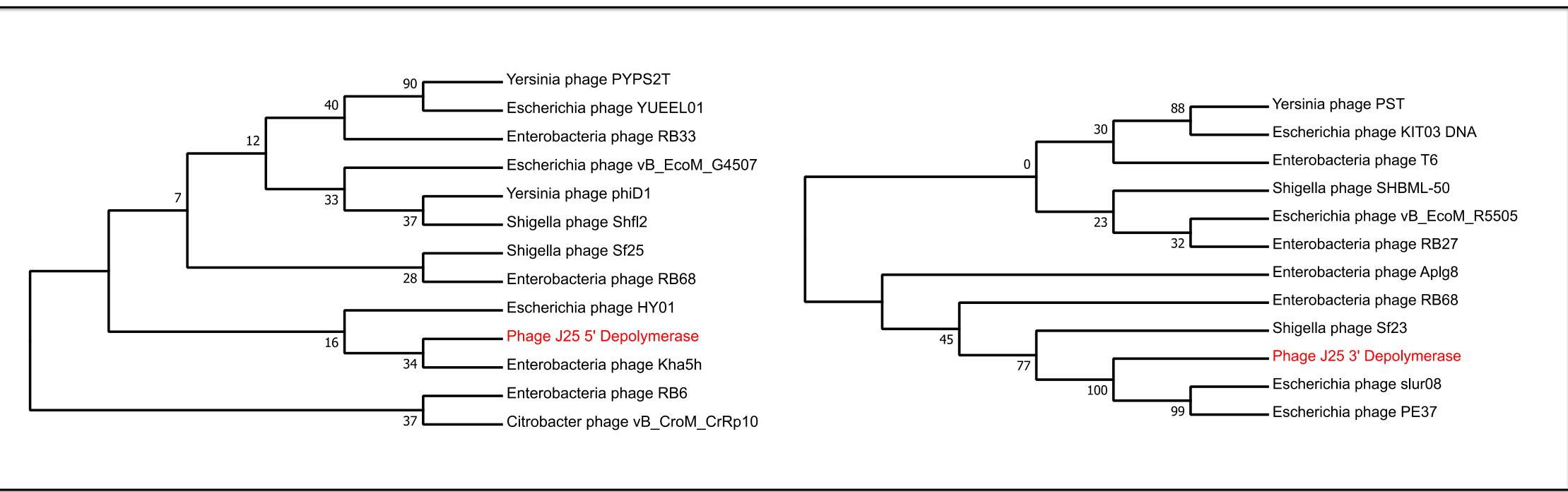
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Myoviridae family, Photo credit P.K. Litt





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References: 1. Altschul, S. F., et al. (1990). "Basic local alignment search tool." J Mol Biol 215(3): 403-410. 2. Birge, E. A. (2006). Bacterial and Bacteriophage Genetics. New York, Springer-Verlag. 3. Keen EC. (2015) A century of phage research: bacteriophages and the shaping of modern biology. Bioessays.37(1):6-9. 4. Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870-1874. 5. Pires, D. P., et al., (2016). Bacteriophage-encoded depolymerases: their diversity and biotechnological applications. Appl. Microbiol. Biotechnol. 100: 2141-2151. 6. Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425. 7. Snyder, Larry, Peters, J. E., Henkin, Tina M. and Champness, Wendy (2013). Molecular Genetics of Bacteria, ASM Press. 8. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA) 101:11030-11035.

Phage J25 Phylogeny based on Depolymerase Gene Sequences

The evolutionary history was inferred using the Neighbor-Joining method [5]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [9]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [8] and are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA7 [4].

Conclusion:

Identified Depolymerase-like gene in genomes similar to J25 • Designed PCR primers, amplified the gene

Sequenced entire J25 Depolymerase-like gene and flanking regions Used phylogeny to identified closely related bacteriophages to J25 • Phage J25 is most closely related to group of phage that infects Salmonella, Shigella, E. coli and Enterobacter

9. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.





60 Strongly Basic(+) Amino Acids (K,R) 73 Strongly Acidic(-) Amino Acids (D,E) 165 Hydrophobic Amino Acids (A,I,L,F,W,V) 156 Polar Amino Acids (N,C,Q,S,T,Y)