

Introduction: *What is a bacteriophage?*

An agent that parasitizes a bacterium, reproduces inside, then lyses the bacterium^{3,8}. Bacteriophages are viruses; there are more than 10^{31} bacteriophage in the world⁵.

What is Salmonella and EHEC food poisoning?

Each year, salmonella infections, called salmonellosis, sicken more than 1 million people. Escherichia coli (E. coli) is a bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E.coli* strains are harmless, but some, such as enterohemorrhagic *E. coli* (EHEC) can cause serious disease.

Treatment with Bacteriophages -

Wash leafy greens, equipment, etc. to reduce bacterial contamination Collaborators at OSU Stillwater have isolated many anti-salmonella or anti-EHEC bacteriophage. Nothing is known about them.

Purpose:

- Clone genomic fragments of bacteriophage P16 and sequence DNA
- Use bioinformatics to determine the identity/type of \bullet bacteriophage P16 using DNA sequence
- Similar genomes can be used to design PCR primers to amplify important genes from P16

Materials and Methods:

Bacteriophage P16 was obtained from Dr. Jaroni at Animal and Food Sciences, OSU Stillwater Phage DNA was cleaved by sonication and cloned into pBluescript II SK $[+]^{1,7}$. DNA sequencing was performed at the OSU Core facility, Stillwater, OK.

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 GenBank². MEGA7 was used to align, compare, and create a phylogeny on the DNA sequence^{4, 7}.



Siphoviridae family Photo credit P.K. Litt fl (+) origin 135-441 β-galactosidase α-fragment 460–816 multiple cloning site 653-760 lac promoter 817–938 pUC origin 1158-1825 ampicillin resistance (bla) ORF 1976-2833

pBluescript II SK (+/-) Multiple Cloning Site Region (sequence shown 598-826)

TTGTAAAACGACGGCCAGTGAGCGCGCGTAATACGACTCACTATAGGGCGAA' M13 -20 primer binding site

... GGTATCGATAAGCT KS primer binding site

3 Promoter

Cloning, Sequencing, and Identification of Phage 16, an Unknown Salmonella or EHEC (Enterohemorrhagic E. coli) Bacteriophage

1. Union High School, Tulsa, OK 2. Holland Hall, Tulsa, OK

Results:



This image shows the number of cases in each state as of June 27, 2018.



Conclusions

Phage 16 is most similar to T5-like phage "chee" 130 1-ASU and bacteriophage that infect Salmonella such as phage Stitch

References:

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Clone 10,233 appears to have an insert



The evolutionary history was inferred by using the Maximum Likelihood method based on the JTT matrix-based model⁴. The tree with the highest log likelihood is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 166 positions in the final dataset. Evolutionary analyses were conducted in MEGA7⁶.





Phage 16 Clone 10,233

DNA sequence is 504 bp Part of a conserved bacteriophage gene Homolog of tail protein gene