



Complete Genome Sequences of Two *Escherichia coli* O157:H7 Phages Effective in Limiting Contamination of Food Products

Yingying Hong, Yanying Pan, Nicholas J. Harman, Paul D. Ebner

Department of Animal Sciences, Purdue University, West Lafayette, Indiana, USA

We previously demonstrated that application of bacteriophages significantly reduced *Escherichia coli* O157:H7 contamination in spinach and ground beef. Here, we present the genomic sequences of two bacteriophages, vB_EcoS_FFH_1, a T5-like phage, and vB_EcoM_FFH_2, an rV5-like phage, used in those treatments.

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Address correspondence to Paul D. Ebner, pebner@purdue.edu.

*E*scherichia coli O157:H7 is a shiga toxin-producing food-borne pathogen that results in over 60,000 illnesses each year in the United States alone (1). We have employed bacteriophages to limit *Salmonella* transmission in swine (2) and recently demonstrated that application of lytic bacteriophages to ground beef and spinach significantly reduced *E. coli* O157:H7 contamination (3). We selected two *E. coli* phages (vB_EcoS_FFH_1 [siphovirus] and vB_EcoM_FFH_2 [myovirus]) for genomic sequencing based on their broad spectrum and lytic capacity.

Phage DNA was purified from polyethylene glycol (PEG)precipitated lysates and sequenced via pyrosequencing (454; Eurofins MWG Operon, Huntsville, AL) and sequences were assembled *de novo* using Newbler (version 2.6). Coding DNA sequences (CDSs) were predicted using Glimmer 3.0 (4) and annotation was performed using BLASTp for homology searching in the nonredundant protein sequences database in GenBank (5). tRNA genes were predicted using both tRNAscan-SE 1.21 (6) and ARAGORN (7). Terminal redundant ends (vB_EcoM_FFH_2) were identified using Tandem Repeat Finder (8).

The genome of vB_EcoS_FFH_1 has a length of 108,483 bp and a G+C content of 39.24%. Whole genome alignment revealed that vB_EcoS_FFH_1 showed 87% homology to T5 (GenBank accession no. AY543070) and therefore was classified as a T5-like phage. A total of 160 CDSs and 24 tRNA genes were predicted. Similar high numbers of tRNA genes are found in T5. Of the CDSs, 52 matched proteins with known functions, while 96 encoded previously identified hypothetical proteins. Twelve CDSs did not match any proteins in the NCBI non-redundant protein database. We identified putative Rz and Rz1 genes based on Summer et al. (9). Highly similar sequences are also present in T5, but are not annotated in the three GenBank T5 complete genomes and other available T5-like phage genomes. One section (79,918 to 84,241) of the vB_EcoS_FFH_1 genome appeared largely absent from the three GenBank annotated T5 genomes, but present in the bV_Eco-S_AKFV33 genome (another T5-like phage). Two putative tail fiber proteins and one hypothetical protein were identified in this section.

The genome of vB_EcoM_FFH_2 has a length of 139,020 bp and a G+C content of 43.61%. Whole-genome alignment revealed that vB_EcoM_FFH_2 shared 93% nucleotide homology to *E. coli* phage rV5 (GenBank accession no. DQ832317) indicating that vB_EcoM_FFH_2 is an rV5-like phage. A total of 220 CDSs and 6 tRNA genes were predicted. Of the CDSs, 57 matched proteins with known functions, while 156 matched previously identified hypothetical proteins. Seven CDSs were not homologous to any existing proteins in the NCBI non-redundant protein database. Several complete genomes of rV5-like viruses are now available. The viruses share a high number of proteins, but based on whole-genome comparisons, two rV5-like sub-groups may exist, rV5 and *Salmonella* phage PVP-SE1 (10–12). vB_EcoM_FFH_2 has significantly more sequence similarity to rV5 (both of which were isolated using *E. coli* O157:H7), which would make it a member of the rV5 sub-group.

Nucleotide sequence accession numbers. The complete sequences of vB_EcoS_FFH_1 and vB_EcoM_FFH_2 were deposited in GenBank under the accession numbers KJ190157 and KJ190158, respectively.

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REFERENCES

- Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. 2011. Foodborne illness acquired in the United States. Emerg. Infect. Dis. 17:1339–1340. http://dx.doi.org/10.3201/eid1707.110572.
- Wall SK, Zhang J, Rostagno MH, Ebner PD. 2010. Phage therapy to reduce preprocessing *Salmonella* infections in market-weight swine. Appl. Environ. Microbiol. 76:48–53. http://dx.doi.org/10.1128/AEM.00785-09.
- Hong Y, Pan Y, Ebner PD. 2014. Meat science and muscle biology symposium: development of bacteriophage treatments to reduce *Escherichia coli* O157:H7 contamination of beef products and produce. J. Anim. Sci. 92:1366–1377. http://dx.doi.org/10.2527/jas.2013-7272.
- Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res. 27: 4636–4641. http://dx.doi.org/10.1093/nar/27.23.4636.
- Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Sayers EW. 2012. GenBank. Nucleic Acids Res. 40:D48–D53. http://dx.doi.org/10.1093/ nar/gkr1202.
- 6. Schattner P, Brooks AN, Lowe TM. 2005. The tRNAscan-SE, snoscan

and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res. 33:686-689. http://dx.doi.org/10.1093/nar/gki366.

- 7. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res. 32:11-16. http://dx.doi.org/10.1093/nar/gkh152.
- Benson G. 1999. Tandem repeats finder: a program to analyze DNA se-8. quences. Nucleic Acids Res. 27:573-580.
- Summer EJ, Berry J, Tran TA, Niu L, Struck DK, Young R. 2007. Rz/Rz1 9 lysis gene equivalents in phage of Gram-negative hosts. J. Mol. Biol. 373: 1098–1112. http://dx.doi.org/10.1016/j.jmb.2007.08.045. 10. Truncaite L, Simoliunas E, Zajanckauskaite A, Kalinene L, Mankevi-

ciute R, Staniulis J, Klausa V, Meskys R. 2012. Bacteriophage vB_EcoM_FV3: a new member of "rV5-like viruses." Arch. Virol. 157: 2413-2435. http://dx.doi.org/10.1007/s00705-012-1449-x.

- 11. Kim H, Heu S, Ryu S. 18 June 2014. Complete genome sequence of enterobacteria phage 4MG, a new member of the subgroup "PVP-SE1-like phage" of the "rV5-like viruses." Arch. Virol. http://dx.doi.org/10.1007/ s00705-014-2140-1.
- 12. Kropinski AM, Waddell T, Meng J, Franklin K, Ackermann HW, Ahmed R, Mazzocco A, Yates J, Lingohr EJ, Johnson RP. 2013. The host-range, genomics and proteomics of Escherichia coli O157:H7 bacteriophage rV5. Virol. J. 10:6. http://dx.doi.org/10.1186/1743-422X-10-76.