

Complete Genome Sequence of *Salmonella enterica* Serovar Typhimurium Bacteriophage SPN3UB

Ju-Hoon Lee,^b Hakdong Shin,^a and Sangryeol Ryu^a

Department of Food and Animal Biotechnology and Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul, South Korea^a; and Department of Food Science and Biotechnology, CHA University, Seongnam, South Korea^b

***Salmonella* is one of the major pathogenic bacteria that cause food poisoning. To elucidate the host infection mechanism of *Salmonella enterica* serovar Typhimurium-targeting phages, the bacteriophage SPN3UB was isolated from a chicken fecal sample. This phage belongs morphologically to the *Siphoviridae* family and infects the host via the O antigen of lipopolysaccharide (LPS). To further understand its infection mechanism, we completely sequenced and analyzed the genome. Here, we announce its complete genome sequence and report major findings from the genomic analysis results.**

Salmonella is a pathogenic bacterium that causes salmonellosis via contaminated foods (6, 7). To develop phages as a biocontrol agent for controlling this pathogen in foods, understanding of the host infection mechanism of *Salmonella* phages is important (2, 3, 10). While the O antigen of lipopolysaccharides (LPS) is a common host receptor for infection in the *Myoviridae* (FelixO1) (11) and *Podoviridae* (P22, ε34) families (4, 13, 14), phages in the *Siphoviridae* family that use this host receptor, such as SETP3, are rare (8). The *S. Typhimurium*-targeting SPN3UB phage, which belongs to the *Siphoviridae* family, could not infect the *rfaL* (O-antigen ligase)-deficient mutant strain of *S. Typhimurium* SL1344 (data not shown), suggesting that it infects the host via the O antigen of LPS (12). To further understand its host infection mechanism, we completely sequenced and analyzed the genome.

Phage genomic DNA was isolated using the standard alkaline lysis method (15) and was sequenced using a Genome Sequencer FLX Titanium instrument by Macrogen (Korea). Assembly of quality filtered reads was performed using a 454 Newbler 2.3 assembler, and open reading frames (ORFs) were predicted using GeneMarkS (5), Glimmer 3.02 (9), and FgenesV (Softberry, Inc., Mount Kisco, NY). Ribosomal binding sites were confirmed using RBSfinder (J. Craig Venter Institute, Rockville, MD). Annotation of the predicted ORFs was conducted using BLASTP (1) and InterProScan (16).

Phage SPN3UB has a double-stranded DNA (dsDNA)-based genome consisting of 47,355 bp with a GC content of 49.61% and 71 ORFs but no tRNA, indicating that it is the largest genome sequence in the *Siphoviridae* phage family that uses the O antigen of *Salmonella* LPS as a host receptor. This phage genome encodes head/tail structure proteins (major capsid protein, tape measure protein, minor tail proteins M and L, tail assembly proteins K and I, and tail fiber protein J), phage packaging terminases (terminase large and small subunits), integration and recombination protein and enzymes (integrase, excisionase-like protein, RecT recombinase, and RecE exodeoxyribonuclease), lysogeny control proteins (Cro, CI, and CII), phage replication proteins (PrpO replication protein and DnaC DNA replication protein), antitermination proteins (antitermination protein Q), host cell lysis enzyme and peptidases (endolysin and Rz/Rz1 endopeptidases), and proteins for additional functions (Arc-like DNA binding protein, antirepressor family protein, Eaa protein, DinI DNA damage-inducible protein, NinG, and Kila-N domain protein). Because this phage

has only one tail fiber J protein, it may play an important role in host infection via the O antigen of LPS. The lysogeny control proteins and antitermination Q protein may contribute to the formation of lysogen during infection. Reconstruction of the phage from the lysogen was confirmed by mitomycin C induction (data not shown). Interestingly, some of the replication proteins, such as helicase, primase, etc., are missing. It is likely that this phage takes advantage of host replication proteins or that they are annotated to hypothetical proteins due to a too-low level of identity with other phage replication proteins in the GenBank database. The complete genome sequence of *S. Typhimurium* SPN3UB phage provides extended information about host infection and interaction mechanisms with this phage.

Nucleotide sequence accession number. The complete genome sequence of *S. Typhimurium* bacteriophage SPN3UB is available in GenBank under accession number [JQ288021](https://www.ncbi.nlm.nih.gov/nuccore/JQ288021).

ACKNOWLEDGMENTS

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (20090078983). H. Shin was the recipient of a graduate fellowship provided by the Ministry of Education, Science and Technology through the Brain Korea 21 Project.

REFERENCES

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
2. Andreatti Filho RL, et al. 2007. Ability of bacteriophages isolated from different sources to reduce *Salmonella enterica* serovar enteritidis in vitro and in vivo. *Poult. Sci.* 86:1904–1909.
3. Atterbury RJ, et al. 2007. Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. *Appl. Environ. Microbiol.* 73:4543–4549.
4. Baxa U, et al. 1996. Interactions of phage P22 tails with their cellular receptor, *Salmonella* O-antigen polysaccharide. *Biophys. J.* 71:2040–2048.
5. Besemer J, Lomsadze A, Borodovsky M. 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Im-

Received 27 December 2011 Accepted 29 December 2011

Address correspondence to Sangryeol Ryu, sangryu@snu.ac.kr.

Copyright © 2012, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JVI.07226-11

- plications for finding sequence motifs in regulatory regions. *Nucleic Acids Res.* 29:2607–2618.
6. Centers for Disease Control and Prevention. 2007. Bacterial foodborne and diarrheal disease national case surveillance. Annual report 2005. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, GA.
 7. Centers for Disease Control and Prevention. 2008. *Salmonella* surveillance. Annual summary 2006. Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, GA.
 8. De Lappe N, Doran G, O'Connor J, O'Hare C, Cormican M. 2009. Characterization of bacteriophages used in the *Salmonella enterica* serovar Enteritidis phage-typing scheme. *J. Med. Microbiol.* 58:86–93.
 9. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679.
 10. Greer GG. 2005. Bacteriophage control of foodborne bacteria. *J. Food Prot.* 68:1102–1111.
 11. MacPhee DG, Krishnapillai V, Roantree RJ, Stocker BA. 1975. Mutations in *Salmonella typhimurium* conferring resistance to Felix O phage without loss of smooth character. *J. Gen. Microbiol.* 87:1–10.
 12. Park M, et al. 2011. Characterization and comparative genomic analysis of a novel bacteriophage, SFP10, simultaneously inhibiting both *Salmonella enterica* and *Escherichia coli* O157:H7. *Appl. Environ. Microbiol.* 78:58–69.
 13. Venza Colon CJ, Vasquez Leon AY, Villafane RJ. 2004. Initial interaction of the P22 phage with the *Salmonella typhimurium* surface. *P. R. Health Sci. J.* 23:95–101.
 14. Villafane R, Zayas M, Gilcrease EB, Kropinski AM, Casjens SR. 2008. Genomic analysis of bacteriophage epsilon 34 of *Salmonella enterica* serovar Anatum (15+). *BMC Microbiol.* 8:227.
 15. Wilcox SA, Toder R, Foster JW. 1996. Rapid isolation of recombinant lambda phage DNA for use in fluorescence *in situ* hybridization. *Chromosome Res.* 4:397–398.
 16. Zdobnov EM, Apweiler R. 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. *Bioinformatics* 17: 847–848.