Complete Genome Sequence of *Salmonella* Bacteriophage SPN3US

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Salmonella **bacteriophage SPN3US was isolated from a chicken fecal sample. It is a virulent phage belonging to the** *Myoviridae* **family and showing effective inhibition of** *Salmonella enterica* **and a few** *Escherichia coli* **O157:H7 strains. Here we announce the completely sequenced first genome of a** *Salmonella* **phage using flagella as receptors. It is the largest genome among** *Salmonella* **phages sequenced to date, and major findings from its annotation are described.**

Salmonellosis is one of the most serious diseases caused by food-borne pathogens (8). While antibiotics have been broadly used to treat this disease, emergence of antibiotic resistance in *Salmonella* is getting more problematic (4). Bacteriophage treatment is now considered a good alternative biocontrol method to inhibit this pathogen (9). Because bacteriophage treatment gained the status of "generally recognized as safe" by the U.S. FDA in 2006 (3), the phage therapy approach could be useful for inhibiting pathogenic *Salmonella*.

Salmonella bacteriophage SPN3US was isolated from chicken feces using the host strain *Salmonella enterica* serovar Typhimurium LT2. A receptor study revealed that this phage infects *Salmonella* using flagella as receptors (data not shown). Although flagella were previously reported as phage receptors (6), the complete genome sequence of the *Salmonella* flagellum-targeting phage has never been reported yet.

Genomic DNA was extracted from the stock by the alkaline lysis method (11). A pyrosequencing approach was used with the Genome Sequencer FLX System Titanium series by Macrogen in Korea (55× coverage), and the quality filtered reads were assembled into a complete genome sequence using the 454 Newbler 2.3 assembler. Prediction of open reading frames (ORFs) was performed using the GAMOLA automatic annotation program (1), and predicted ORFs were confirmed using the Glimmer 3.02 (5), GeneMark.hmm (7), and FgenesV (Softberry, Inc., Mount Kisco, NY) software. Annotation of predicted ORFs was conducted using the results of BLASTP (2) and InterProScan (12) analyses.

The complete genome of phage SPN3US revealed a length of 240,413 bp with a G+C content of 48.54%, 264 ORFs, and two tRNAs, suggesting the largest *Salmonella* phage genome found to date (10). The average gene length is 855 bp, and the gene density is 1.098/kb. Of the ORFs, 87.5% are positioned on one of two DNA strands.

While the gene coding percentage is 93.9% in the genome, 79.2% of the ORFs were annotated as hypothetical, probably due to insufficient database information about the functional genes of *Salmonella* phage genomes. This genome contains functional genes related to phage structure and packaging (major capsid protein, unknown phage structure proteins, and terminase), tail structure for host interaction (tail fiber protein, tail sheath protein, and tail-associated protein), replication/transcription (helicase, DNA-directed RNA polymerases, SbcCD nuclease, endo-DNase, RNase H, and transcription regulator), host lysis (endolysin without holin), and additional functions (phage DNA adenine methylase for protection from host restriction-modification systems and dihydrofolate reductase/thymidylate kinase/thymidylate synthase, probably for folate metabolism). However, these functional genes are not positioned in the same gene clusters but are scattered over the genome. Interestingly, the repeats of six RNA polymerase beta subunits suggest that transcription of phage genes may be dominant, rather than gene transcription of the host. This phage genome has only one phage fiber protein, which probably interacts with the host flagella for infection. The complete genome analysis of this phage provides a new insight into its characteristics and interactions with *Salmonella*.

Nucleotide sequence accession number. The complete genome sequence of *Salmonella* phage SPN3US is available in GenBank under accession number JN641803.

REFERENCES

1. **Altermann, E., and T. R. Klaenhammer.** 2003. GAMOLA: a new local solution for sequence annotation and analyzing draft and finished prokaryotic genomes. OMICS **7:**161–169.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Cairns, B. J., and R. J. H. Payne.** 2008. Bacteriophage therapy and the mutant selection window. Antimicrob. Agents Chemother. **52:**4344–4350.
4. **CDC.** 2008. *Salmonella* surveillance: annual summary 2006. Centers for Disease Control and Prevention, Atlanta, GA.
5. **Delcher, A. L., K. A. Bratke, E. C. Powers, and S. L. Salzberg.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics **23:**673–679.
6. **Kagawa, H., N. Ono, M. Enomoto, and Y. Komeda.** 1984. Bacteriophage chi sensitivity and motility of *Escherichia coli* K-12 and *Salmonella typhimurium* Fla⁻ mutants possessing the hook structure. J. Bacteriol. **157:**649–654.

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7. **Lukashin, A. V., and M. Borodovsky.** 1998. GeneMark.hmm: new solutions for gene finding. Nucleic Acids Res. **26:**1107–1115.

8. **Mead, P. S., et al.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.

9. **O'Flaherty, S., R. P. Ross, and A. Coffey.** 2009. Bacteriophage and their lysins for elimination of infectious bacteria. FEMS Microbiol. Rev. **33:**801–819.

10. **Pickard, D., et al.** 2010. A conserved acetyl esterase domain targets diverse bacteriophages to the Vi capsular receptor of *Salmonella enterica* serovar Typhi. J. Bacteriol. **192:**5746–5754.

11. **Wilcox, S. A., R. Toder, and J. W. Foster.** 1996. Rapid isolation of recombinant lambda phage DNA for use in fluorescence *in situ* hybridization. Chromosome Res. **4:**397–398.

12. **Zdobnov, E. M., and R. Apweiler.** 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. Bioinformatics **17:**847–848.