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Complete Genome Sequence of Bacteriophage SSU5 Specific for *Salmonella enterica* serovar Typhimurium Rough Strains

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Salmonella enterica serovar Typhimurium rough strain-specific phage SSU5 was isolated, and its whole genome was sequenced. The 103,229-bp-long double-stranded DNA genome of SSU5 encodes 130 open reading frames with one tRNA for asparagine. Genomic analysis revealed that SSU5 might be the phylogenetic origin of cryptic plasmid pHCM2 harbored by *Salmonella* Typhi CT18.

*S*almonella sp. is an enteric pathogen that causes about 42,000 cases of reported salmonellosis and, consequently, approximately 400 deaths in the United States annually (3). To develop bacteriophage cocktails as an alternative biocontrol agent for *Salmonella enterica* serovar Typhimurium, several *S.* Typhimurium-specific phages have been isolated by our group. Interestingly, most of them use only one of three types of host receptor; outer membrane protein BtuB, O-antigen, and flagella (unpublished data). By using an O-antigen and BtuB doubly deficient mutant of *S.* Typhimurium strain LT2 as the host bacterium, we isolated the *Siphoviridae* family phage SSU5, which infects only rough strains of *S.* Typhimurium by using the lipopolysaccharide (LPS) core antigen as a receptor.

The genomic DNA of SSU5 was extracted by manual phenol-chloroform extraction (8) and subjected to pyrosequencing using the Genome Sequencer FLX Titanium from Macrogen, Seoul, South Korea. GS De Novo assembler (v. 2.60) was used to assemble quality filtered reads, and GeneMarkS (2), Glimmer 3.02 (4), and FgenesV (Softberry, Inc., Mount Kisco, NY) were used to predict open reading frames (ORFs) that encode proteins of more than 40 amino acids. The predicted ORFs were annotated on the basis of the results obtained with BLASTP (1), InterProScan (9), and the NCBI conserved domain database (7). tRNAs were predicted by tRNAscan-SE (6).

The genome of SSU5 was a linear double-stranded DNA consisting of 103,229 bp with a G+C composition of 51.11%. A total of 130 ORFs and one tRNA for asparagine were predicted. More than half ($n = 72$) of the ORFs were annotated as hypothetical proteins, indicating the novelty of SSU5. The other ORFs encoded proteins related to DNA packing/morphogenesis (terminase large subunit, major capsid protein, minor tail protein, etc.), DNA replication/repair (DNA helicase, primase, ligase, DNA polymerases, exonucleases, recombinase, etc.), host lysis (holin, endolysin, and Rz1), lysis/lysogeny switch (phage repressor and antirepressor), and other functions (repressor of phase I flagellin, selenium-binding protein YdfZ, etc.). A putative receptor-recognizing phage tail fiber adhesin that might interact with the *Salmonella* LPS core antigen was predicted.

Interestingly, the genome of SSU5 showed a high level of homology to cryptic plasmid pHCM2 (106,516 bp; NC_003385) harbored by *Salmonella* Typhi strain CT18 (5). Comparative genomic analysis revealed that all except 22 of the ORFs of SSU5 showed up to 100% identity with ORFs of pHCM2 at the protein level. The 22 ORFs showing no homology to ORFs of pHCM2 included some key elements for phage life cycle (i.e., receptor-recognizing phage tail fiber adhesin, superinfection exclusion protein, and phage repressor), whereas a putative phage integrase (NP_569492.1) was found in pHCM2 but not in SSU5, suggesting that cryptic plasmid pHCM2 might have originated from phage SSU5 or its ancestors.

Analysis of the complete genome of SSU5 revealed a substantial distinction of SSU5 from other known phages, which supports the value of SSU5 as a phage cocktail component. Also, it would provide an insight into the phylogenetic relationship between phages and cryptic plasmids.

Nucleotide sequence accession number. The sequence of the *S.* Typhimurium phage SSU5 genome is available at GenBank under accession number JQ965645.

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