

Complete Genome of *Citrobacter freundii* Siphophage Stevie

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***Citrobacter freundii* is an opportunistic pathogen responsible for many urinary tract infections acquired in hospitals and is thus a concern for public health. *C. freundii* phage Stevie might prove beneficial as a treatment against these infections. The complete genome of Stevie and its key features are described here.**

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Citrobacter, a Gram-negative bacteria, includes many opportunistic pathogens. *C. freundii* causes urinary and respiratory tract infections, many of which have been found to be resistant to a number of antibiotics (1, 2). This makes bacteriophages infecting *C. freundii*, such as siphophage Stevie, described here, of particular interest for combating multi-drug resistance (3).

Bacteriophage Stevie was isolated from a dirt sample collected in College Station, TX. Phage DNA was sequenced in an Illumina MiSeq 250-bp paired-end run with a 550-bp insert library at the Genomic Sequencing and Analysis Facility at the University of Texas (Austin, TX). Quality-controlled trimmed reads were assembled to a single contig at 20.9 fold coverage using Velvet version 1.2.10. Genes were predicted using GeneMarkS (4) and corrected using software tools available on the Center for Phage Technology (CPT) Galaxy instance (<https://cpt.tamu.edu/galaxy-public/>) (5–7). Morphology was determined using transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center.

Stevie is a T1-like phage with a 49,759-bp partially permuted genome, G+C content of 43.7%, 91.5% coding density, and 91 predicted coding sequences. Stevie shares 47.7, 62.8, and 44.2 percent nucleotide sequence identity to siphophages T1 (NC_005833), TLS (NC_009540), and FSL SP-030 (NC_021779), respectively, as determined by Emboss Stretcher (8). It has an abundance of rho-independent terminators, a distinguishing feature of T1-like phages, with 21 predicted in its genome compared to the 17 found in phage T1 (9). Sixteen occurrences of a 22-bp repeat sequence (consensus-VWATAGCAYKWWTTGYTAA AAV) were identified in the sequence of Stevie. A comparable repeat with 20 occurrences was identified in the T1 genome (9). The repeat sequence is asymmetric, predominantly intergenic, and oriented in one direction with respect to the direction of transcription. A 13-bp repeat with similar features was described in mycobacteriophage L5 as a stopoperator involved in the maintenance of transcriptional silence of the integrated prophage (10). The role these repeats play in the lytic lifestyle of T1 and Stevie is unknown.

Genes encoding T1-like morphogenesis proteins include the

prohead protease, major capsid protein, tail fibers, tail assembly proteins, tape measure protein, and tape measure chaperones (complete with a conserved translational frameshift) (11). Few DNA replication/recombination proteins were identified (single-strand annealing protein, single-strand binding protein, primase, helicase, and an endonuclease). Stevie encodes a DNA (N⁶-adenine)-methyltransferase and presumably methylates its DNA-like phage T1 (12, 13). Stevie uses a headful DNA-packaging mechanism as determined by homology of TerL to large terminase proteins of phages with known *pac*-type strategies (14).

Similar to T1-like phage TLS, Stevie encodes a T4 Stp-like peptide that protects the phage from specific host restriction enzymes (15). In T4, the Stp-mediated restriction enzyme protection activates a host tRNA^{Lys}-specific anticodon nuclease (ACNase), the action of which is mitigated by phage encoded polynucleotide kinase, also present in the Stevie genome. The lysis cassette consists of a predicted (class-II) pinholin (6), a R²¹-like signal anchor release (SAR) lysozyme (16, 17), and a unimolecular spanin (18).

Nucleotide sequence accession number. The genome sequence of phage Stevie was deposited in GenBank under the accession no. [KM236241](https://ncbi.nlm.nih.gov/nucl/KM236241).

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