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Complete Genome Sequences of *Mycobacterium smegmatis* Phages SVoro and PrinceCharming

Kristi M Westover[1*], Kailyn C Cabana[1*], Simon SE Cherry[1*], Norah B Kay[1*], Saachi Mehra[1*], Olivia L Morgan[1*], Ash H Olivet[1*], Samuel W Reese[1*], Sasha J Vorontsov[1*], Ella A Williams[1*], Mohammed RS Zyara[1*], Victoria J Frost[1§*]

[1]Department of Biology, Winthrop University, Rock Hill, SC, United States

[§]To whom correspondence should be addressed: frostv@winthrop.edu

[*]These authors contributed equally.

Abstract

SVoro and PrinceCharming were isolated from moist soil on the campus of Winthrop University and isolated on the host bacterium *Mycobacterium smegmatis* mc^2 155. SVoro has a myovirus morphology and a genome of 155,109 bp, while PrinceCharming exhibits siphovirus morphology and a genome of 67,429 bp. Lysogens could not be raised for SVoro or PrinceCharming, suggesting these are virulent phages. SVoro is classified in the C1 subcluster, and PrinceCharming belongs to the B2 subcluster.

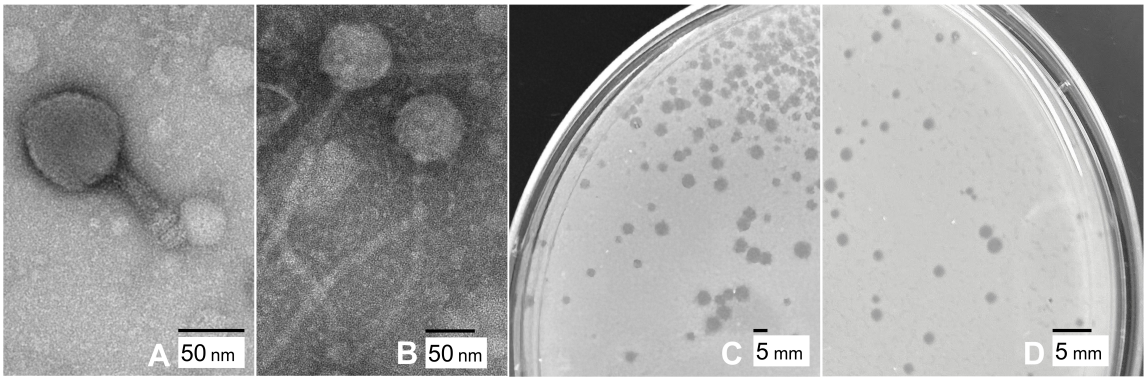


Figure 1. Particle and plaque morphologies for SVoro and PrinceCharming:

Transmission electron micrographs of mycobacterium phages SVoro (A) and PrinceCharming (B). Phage lysates were negatively stained with 1% uranyl acetate, and images taken with a JEOL JEM-1230 TEM at 100kV acceleration voltage. SVoro (C) forms clear plaques (5-8 mm, n=5). PrinceCharming (D) forms relatively small, clear plaques (1-2 mm, n=4).

Description

There is a growing need for alternative treatments of antibiotic-resistant bacterial infections as they become more difficult to manage (Chinemerem Nwobodo et al., 2022). Using bacteriophages therapeutically is a promising option. Phage therapy has demonstrated positive clinical outcomes in treating bacterial infections caused by multidrug-resistant pathogens in immunocompromised patients, including after organ transplantation (Green et al., 2023), in burn wound cases (Teney et al., 2024), and in carbapenem-resistant *Pseudomonas aeruginosa* lung infections (Yang et al., 2025). In the report described here, two mycobacteriophages, SVoro and PrinceCharming, were extracted from soil near tree roots in the Hardin Garden at Winthrop University, Rock Hill, South Carolina (see Table 1 for GPS coordinates). A buffer (10% glycerol, 1 mM $CaCl_2$) was added to the samples, then shaken for 24 hours at 24° C to loosen soil particles. After settling, the liquid was filtered (0.22 µm) and enriched with *Mycobacterium smegmatis* mc^2 155 (*M. smegmatis*) in 7H9 liquid medium for four days, with shaking (250 rpm at 37° C). Following re-filtration, standard plaque assay procedures (Zorawik et al., 2024) were used to purify and amplify both phages. This involved mixing phage lysates with molten 7H9 agar and the bacterial host *M. smegmatis*, overlaying the mixture on 7H9 agar plates, and incubating for 2 days at 37° C. The plaques for SVoro were clear and relatively large (5-8 mm, n=5), while PrinceCharming's plaques were small (1-2 mm, n=4) but also clear, indicating a lytic lifecycle for both phages. This was supported by the inability of either phage to produce lysogens (Murphy et al., 2025ab). Transmission electron microscopy revealed that SVoro has a short, contractile tail (79-85 nm, n=6) typical of a myovirus morphology, with a relatively large capsid (79-87 nm diameter, n=4), whereas PrinceCharming has a long, flexible tail (221-261 nm, n=5) characteristic of a siphovirus morphology, and a smaller capsid (66-73 nm diameter, n=5).

High-titer lysates from both phages were used to extract genomic DNA with the Wizard DNA Cleanup kit (Promega), and the resulting DNA was sequenced on an Illumina NextSeq 1000 (XLEAP-P1 kit) platform, with libraries prepared using the NEB Ultra II FS kit. Raw 100 base reads were trimmed with cutadapt v4.7 (using the option: –nextseq-trim 30) (Martin 2011), and filtered with skewer v0.2.2 (using the options: -q 20 -Q 30 -n -l 50) (Jiang et al., 2014), then assembled with Unicycler v0.5.1 and Consed v29 (Wick et al., 2017; Gordon and Green 2013). Sequencing results and features of the phage genome are detailed in Table 1, which presents the predicted gene count and the phage cluster assignment based on gene content similarity (GCS) of at least 35% to phages in the Actinobacteriophage database (https://phagesdb.org/) (Russell and Hatfull 2017; Pope et al., 2017).

Bioinformatic analyses were conducted using comparative and predictive software with default settings, unless otherwise specified. Sequences were annotated with PECAAN (v20240320) (Rinehart et al., 2016), integrated with Glimmer v3.02 (Delcher et al., 2007), GeneMark v4.28 (Besemer and Borodovsky, 2005), Starterator v558 (http://phages.wustl.edu/starterator/), and Phamerator (Cresawn et al., 2011) using the Actino_draft database (v578). Additional tools included BLAST (Altschul et al., 1990) against the Actinobacteriophage and NCBI non-redundant database, and HHpred v3 (Söding et al., 2005) using PDBmmCIF70, Pfam v.37, and NCBI Conserved Domains databases. Predicted transmembrane domains were analyzed with TOPCONS v2 (Bernsel et al., 2009), DeepTMHMM v1.0.44 (integrated into PECAAN) (Hallgren et al., 2022), and SOSUI v1.11 (Hirokawa et al., 1998). Transfer RNAs were detected using Aragorn v1.2.38 (Laslett and Canback 2004), and v1.1 embedded into DNA Master (Pope and Jacobs-Sera 2018), and tRNAscan-SE v2.0.6 (Lowe and Eddy 1997).

Annotation of SVoro and PrinceCharming revealed that both genomes contain putative genes that encode proteins involved in the structure and assembly of the phage (e.g., terminase, capsid proteins, tape measure proteins, portal proteins), lysis (e.g., lysin A and lysin B), and those involved in recombination and replication (e.g., DNA resolvase, DNA primase/polymerase). Neither phage genome contains genes predicted to encode integrase or repressor functions. Exclusive use of a lytic lifestyle was supported by SVoro's and PrinceCharming's inability to create a lysogen (Murphy et al., 2025ab). SVoro contains many tRNAs in its genome (32), one tmRNA, and genes encoding putative functions such as a PnuC-like nicotinamide riboside transporter and a ThyX-like thymidylate synthase, all typical of C1 phages annotated so far (Cobb et al, 2025). PrinceCharming has the characteristic genome arrangement seen in the B2 cluster, with most genes at the right end of the genome encoded in the reverse direction, and three putative genes involved in queuosine biosynthesis conserved among subcluster B2 phages (Clermont et al., 2024).

Data availability

The complete genome sequences of phages SVoro and PrinceCharming are available in GenBank (accession numbers PV940981 and PV915860, respectively). The raw sequencing reads are available in the NCBI SRA under accession numbers SRX32748213 and SRX32748211, respectively. The Actinobacteriophage sequencing BioProject accession number is PRJNA488469.

TABLE 1 Phage sample locations and genome assembly results

Phage Name	SVoro	PrinceCharming
Sample Location (GPS)	34.9375 N, 81.030556 W	34.93714 N, 81.03081 W
Sequencing Reads	2.3M	1.9M
Sequencing coverage (X)	1443	2662
Genome size (bp)	155,109	67,429
Genome ends	Circularly permuted	Circularly permuted
GC content (%)	64.8	68.9
No. of genes	228	92
No. of tRNAs	32	0
Cluster	C1	B2

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