Genome Sequence of *Mycobacterium abscessus* Phage phiT45-1

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**ABSTRACT** Mycobacteriophage phiT45-1 is a newly isolated bacteriophage spontaneously released from *Mycobacterium abscessus* strain Taiwan-45 that lytically infects *M. abscessus* strain BWH-C; phiT45-1 also infects *M. abscessus* ATCC 19977 but not *Mycobacterium smegmatis*. Phage phiT45-1 has a 43,407-bp genome and carries a polymorphic toxin-immunity cassette associated with type VII secretion systems.

Non-tuberculous mycobacteria (NTM) are mycobacterial species that do not cause tuberculosis or leprosy (1). Among the many NTM pathogens, *Mycobacterium abscessus* is often antibiotic resistant and refractory to treatment. *M. abscessus* infections are frequent among cystic fibrosis patients and those with bronchiectasis and can disseminate in immunosuppressed patients (2, 3). The robust nature of *M. abscessus* contributes to the prevalence of latent infections and the evolution of multidrug-resistant (MDR) strains (1). The rise of antibiotic resistance in *M. abscessus* cases has prompted consideration of mycobacteriophages—viruses that infect mycobacteria—as a therapeutic alternative (4).

It is not uncommon for strains of *M. abscessus* to contain prophages (5), and spontaneous release of phage particles from such strains has been previously described (6). Phage phiT45-1 was isolated by plating culture supernatant from *M. abscessus* Taiwan-45 onto a lawn of *M. abscessus* strain BWH-C (both provided by Chidiebere Akusobi and Eric Rubin) on solid medium at 37°C using standard methods (7). Phage were picked from infected areas, plaque purified, and amplified on BWH-C (7), followed by DNA extraction using the Wizard DNA cleanup system (catalog no. A7280; Promega, Madison, WI). Sequencing libraries were prepared from genomic DNA by using a NEBNext Ultra II FS kit with dual-indexed barcoding. Forty-eight libraries were pooled and run on the Illumina MiSeq platform, yielding 192,000 single-end 150-bp reads and 500-fold coverage of the genome. The raw sequence reads were assembled using Newbler v2.9 with default settings, yielding a single phage contig of 43,407 bp with 65% G+C content. The contig was assessed for completeness, accuracy, and phage genomic termini determination using Consed v.29 as previously described (8); the viral genome sequence has defined ends with 10-base 3’ single-strand extensions. Protein-coding genes were identified using GeneMarkS v4.30 (9), Glimmer v3.02 (10), the Phamerator database Abscessus_phage_and_prophage v3 (11, 12), and DNA Master v5.23.5 (http://cobamide2.bio.pitt.edu) (Fig. 1). Putative functions were assigned to 52% of the 66 protein-coding genes using BLAST (13) and HHpred (14, 15). No tRNA genes were identified by ARAGORN v1.2.41 (16). All tools were run with default parameters unless otherwise stated.

Phage phiT45-1 does not have overall similarity (all BLASTN bit scores of <190) to phages isolated on *M. smegmatis* (17), although its portal, capsid maturation protease, and capsid proteins (4, 5, and 6, respectively; Fig. 1) share >60% amino acid identity with cluster N mycobacteriophages, which have genome sequence lengths similar to that of phiT45-1 (18, 19); like cluster N phages, phiT45-1 also has a siphoviral morphology (family Siphoviridae) (Fig. 1). Early lytic genes include a RecET-like recombination

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system (48 and 49) and several predicted HNH endonucleases (57, 59, 60, 65, and 66; Fig. 1). The presence of a tyrosine integrase (34) and immunity repressor (35) is consistent with phiT45-1 being temperate. Interestingly, phiT45-1 codes for a polymorphic toxin (PT) cassette, including an immunity protein (30), a polymorphic toxin (31) with RipA-like and WXG-100 domains (20, 21), and a WXG-100 protein (32) (22), situated close to the integrase and repressor genes, and likely lysogenically expressed; phiT45-1 gp31 and gp32 are presumably exported via a type VII secretion system. A similar PT system has been reported for M. abscessus phage phiT46-1 (6).

Data availability. Phage phiT45-1 is available at GenBank under accession no. MW570842 and BioProject accession no. PRJNA488469. The sequencing reads are available in the SRA under accession no. SRX10050651.

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