Complete Genome Sequences of *Mycobacterium smegmatis* Phages MelsMeow, Yorick, Virgeve, and Mikro

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**ABSTRACT** *Mycobacterium* phages Mikro, Yorick, Virgeve, and MelsMeow were isolated from soil in Rock Hill, South Carolina. Mikro is a myovirus with a comparatively large genome of 157,166 bp. The remainder are siphoviruses with genome lengths ranging from 59,227 bp to 68,563 bp. All phages were isolated on *Mycobacterium smegmatis*.

Researchers have documented the use of phage therapy to treat multidrug-resistant *Mycobacterium* infections, with promising results (1, 2). Here, *Mycobacterium smegmatis* mc²155 was used to isolate mycobacteriophages from soil at Winthrop University, Rock Hill, SC. Mycobacteriophages Mikro, Yorick, and Virgeve were isolated from damp soil in a shaded flower bed, whereas MelsMeow was isolated from dry soil close to a tree root (see Table 1 for location coordinates [GPS]), using standard procedures (https://seaphages phagediscoveryguide.helpdocsonline.com/home). Soils samples were washed for 2 h using 7H9 broth containing 1 mM CaCl₂ and centrifuged at 4,000 rpm for 10 min, and the supernatant filtered (0.22 μm). A fraction of each filtrate was inoculated with *M. smegmatis* and shaken (250 rpm) at 37°C for 2 to 4 days to enrich for mycobacteriophages before being refiltered. Both enriched and unenriched filtrates were examined for phage by plating in soft agar with *M. smegmatis* and shaking (250 rpm) at 37°C. Mikro was isolated from unenriched filtrate and produced very small (<1 mm diameter) clear plaques. Yorick, Virgeve, and MelsMeow were isolated from enriched filtrates. Virgeve and MelsMeow produced clear plaques, while Yorick produced turbid plaques. Transmission electron microscopy revealed that Mikro has a *Myoviridae* morphotype with a short contractile tail. Phages Yorick, Virgeve, and MelsMeow have *Siphoviridae* morphologies with long flexible tails (Fig. 1).

Phage DNA was extracted using the Wizard DNA cleanup kit (Promega), and libraries were constructed using the NEBNext Ultra II FS DNA library prep kit before sequencing with the Illumina MiSeq v3 platform. Results of the 150-bp single-end raw reads were assembled using Newbler v2.9 and checked for accuracy, coverage, and genomic termini using Consed v29 as previously described (3, 4). Sequencing results and phage genome characteristics are listed in Table 1 and include genome size, GC content, predicted number of genes, and phage cluster designation based on gene content similarity (GCS) of at least 35% to phages within the Actinobacteriophage database (https://phagesdb.org/) using the GCS tool at phagesDB and previously described criteria (5, 6).

Default parameters were used for all bioinformatics analyses. Genome sequences were annotated using DNA Master v5.23.6 (7) embedded with Glimmer v3.02 (8) and GeneMark v2.5.2 (9), Starterator v7 (10), Phamerator v3 (11), HHpred v2.07 (12), and BLASTp v2.13.0 (12). Transfer RNAs were identified using Aragorn v1.1 integrated in DNA Master (7), Aragorn v1.2.38 (13), and tRNAscan-SE v2.0.6 (14).

Annotation revealed putative gene functions for each phage. MelsMeow and Virgeve genomes begin with rightward-transcribed genes that include a portal protein, capsid maturation protease, MuF-like fusion protein, two-tail assembly chaperones, and a tape-measure

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protein. The second portion of these genomes include DNA helicase, DNA Pol I, and HNH endonuclease genes that are transcribed leftwards. Neither genome contains identifiable integrases or immunity repressors, and are predicted to be lytic phages. Yorick’s genome, composed of mostly rightward-transcribed genes, includes an immunity repressor, tyrosine integrase, holin, Cro, and an antirepressor, and is therefore predicted to be temperate, consistent with the turbid plaques it produces. Mikro also contains mostly rightward-transcribed genes, as well as many (32) tRNAs.

**Nucleotide sequence accession numbers.** The complete genome sequences of phages Mikro, Yorick, Virgeve, and MelsMeow are available in GenBank (accession numbers ON456344, ON456356, ON456332, and ON456330, respectively). The raw sequencing reads are available in the NCBI SRA under accession numbers SRX15940724, SRX15940726, SRX14485102, and SRX14483218, respectively. The Actinobacteriophage sequencing BioProject accession number is PRJNA488469.

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**TABLE 1 Phage GenBank and SRA accession numbers and genome assembly results**

| Phage name | Location site (GPS) | Avg coverage (X) | Reads (K) | Cluster | Genome size (bp) | Genome ends | GC content (%) | No. of genes |
|------------|---------------------|------------------|-----------|---------|-----------------|-------------|----------------|--------------|
| Mikro      | 34.940074 N, 81.033099 W | 1,503 | 1,661 | C1 | 157,166 | Circular permuted | 64.8 | 260 |
| Yorick     | 34.940113 N, 81.032921 W | 933 | 385 | F1 | 59,227 | 3’ single-stranded overhang (5’-CGGTAGGCCG-3’) | 61.3 | 99 |
| Virgeve    | 34.940113 N, 81.032921 W | 630 | 658.8 | B1 | 68,046 | Circular permuted | 66.5 | 99 |
| MelsMeow   | 34.93712 N, 81.03197 W | 646 | 620.8 | B1 | 68,563 | Circular permuted | 66.4 | 100 |

**FIG 1** Transmission electron micrographs of *Mycobacterium* phages MelsMeow (A), Yorick (B), Virgeve (C), and Mikro (D). Phage lysates were negatively stained with 1% uranyl acetate.
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