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Isolation and Genome Annotation of MamaT, a Cluster GI Bacteriophage with Podovirus Morphology

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Abstract

MamaT was isolated from a soil sample collected adjacent to an irrigation canal in Pocatello, Idaho, USA. The bacteriophage has a podovirus morphology, infects *Microbacterium foliorum* NRRL B-24224, and has been grouped with bacteriophages in cluster GI based on sequence similarity. The genome size is 16,854 bp with 53.8% GC content. The annotation of the genome provides evidence for 23 putative protein-coding genes, of which 12 have predicted functions based on sequence and structure analysis. While MamaT has no tRNA genes, it appears to have a unique prohead packaging RNA (pRNA) system that includes a putative noncoding pRNA component.

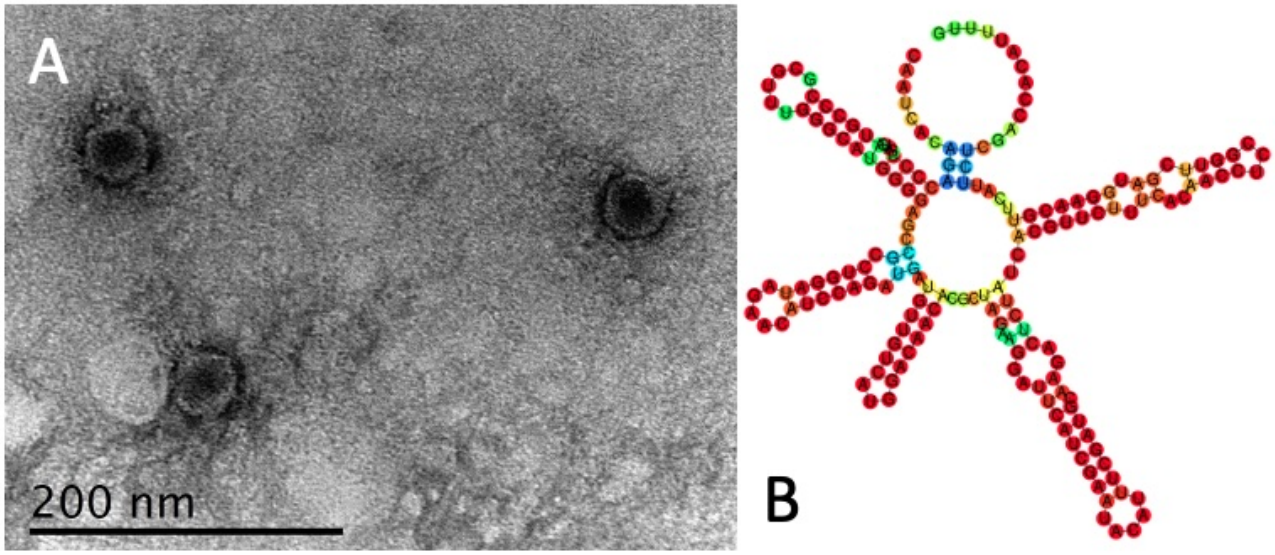


Figure 1. Characterization of MamaT:

A) Podovirus morphology of MamaT with a mean capsid diameter of 35nm +/- 0 nm (n=5 phage particles examined). Image produced by a Zeiss EM900 TEM with an accelerating voltage of 80 kV and 1% uranyl acetate negative staining. B) The minimum free energy (MFE) structure of the putative pRNA fold (located between the stop codon of gp6 and the start codon of gp7), predicted by RNAfold. Colors reflect base-pair probabilities calculated from the partition function (red reflects high confidence).

Description

Bacteriophages are exceedingly diverse but largely under-sampled (Dion et al., 2020). Because of this, great advances in bioengineering and biotherapy are anticipated from the continued discovery of novel phages with unique properties (Mu & Lawley, 2023). Here, we describe the isolation and genetic composition of MamaT, a bacteriophage that infects *Microbacterium foliorum.*

MamaT was isolated from a soil sample consisting of clay and decaying plant material, collected near the Portneuf River at the Price Road wetlands near McCammon, ID (42.622464 N, 112.169022 W) on August 27, 2024, using the following established procedures (Adams et al., 2022; Zorawik et al., 2024). The soil sample was washed with PYCa liquid medium and the supernatant filtered with a 0.22-µm filter. The filtrate was inoculated with *M. foliorum* NRRL B-24224. After incubation with shaking at 30°C for 48 h, the culture was filtered (0.22-mm pore size), and the filtrate was plated using *M. foliorum* PYCa top agar overlays, resulting in plaques after incubation at 30°C for 48 h. Plaques were purified through three additional rounds of plating. A high-titer lysate (>10^{10} pfu/mL) was prepared for DNA sequencing and transmission electron microscopy (TEM). Negative-stain TEM revealed phages with podovirus morphology (Figure 1A). The lack of an identifiable tape measure protein in the genome supports the classification of this phage as a podovirus.

Promega Wizard DNA clean up kit was used to extract genomic DNA from a high-titer lysate. A sequencing library was then prepared using a NEB Ultra II library kit and sequenced on an Illumina NextSeq 1000 (XLEAP-PI kit), resulting in 922,020 single-end 100-bp reads with 1,825-fold coverage. Raw reads were trimmed with cutadapt 4.7 (using the option: –nXextseq-trim 30) and filtered with skewer 0.2.2 (using the options: -q 20 -Q 30 -n -l 50) prior to assembly, yielding a single contig. Consed v29 was used to check for completeness and accuracy and determine phage termini, using default settings (Chung et al., 2017). MamaT's genome length is 16,854 bp with a GC content of 53.8% (notably lower than the host, at 68.7%; Russell et al., 2019). Based on genomic synteny, sequence homology to known protein-priming phages, and an abrupt drop-off in raw sequencing read coverage at the genome boundaries, MamaT is predicted to possess a linear genome capped by covalently bound terminal proteins.

The MamaT genome was auto-annotated by DNAmaster v5.23.6 (http://cobamide2.bio.pitt.edu) using Genemark v2.5 (Besemer and Borodovsky 2005) and Glimmer v3.0 (Delcher et al., 2008) and subsequently manually inspected and refined using PECAAN (http://discover.kbrinsgd.org), Phamerator v454 (Cresawn et al., 2011) for synteny analysis and Starterator (http://phages.wustl.edu/starterator/) to identify start sites by manually comparing to genes within the same protein family (pham) (Horton et al., 2024). To predict putative gene functions, the following tools were deployed: HHpred v2.0 (Soding et al., 2005), using the PDB_mmCIF70, Pfam v.36, and NCBI Conserved Domains databases; BLAST v2.13.0 (Altschul et al. 1990), using the Actinobacteriophage and NCBI non-redundant databases; and Phamerator, using Actino_draft database v578 (cutoff values were 10-7 for BLAST and probability >90% over >50% coverage for HHpred). AlphaFold 3 (Jumper et al. 2021) was used to identify putative function of minor tail proteins. No tRNAs were identified using Aragorn v1.2.41 (Laslett 2004) and tRNAscan-SE v2.0 (Lowe and Eddy 1997). Using default parameters, the annotation process resulted in the identification of 23 putative protein-coding genes, 12 of which could be assigned gene functions. MamaT was assigned to cluster GI based on gene content similarity of at least 35% to phages in the Actinobacteriophage database, PhagesDB (https://phagesdb.org/) (Pope et al., 2017; Russell and Hatfull 2017).

MamaT is the fifth annotated bacteriophage within cluster GI, to date, which includes phages Curie (Horton et al., 2024) and PineapplePizza (LaBianca et al., 2023), and with substantial similarity to *Bacillus* phage φ29 (Xu et al., 2019). Interestingly, phage φ29 has a prohead packaging RNA (pRNA) system that includes a noncoding pRNA component along with genes associated with packaging DNA into the prohead. In MamaT, homologs to these genes include gp15 (terminase) and gp10 (portal protein), and a putative pRNA appears to reside between the stop codon of gp6 and the start codon of gp7 (nucleotide position 4,625 to 4,801). Folded with RNAfold (Gruber et al., 2008), the 177 nt putative pRNA segment is highly consistent with φ29 pRNA in both length (the full-length φ29 pRNA is ~174 nt) and secondary structure: a characteristic 5-way junction with a central hub, a stable closing stem at the 5′/3′ ends, an apical hairpin, and flexible loop regions at appropriate positions within a well-folded, thermodynamically stable RNA (Figure 1B). No conventional upstream promoter was identified in the intergenic space, consistent with the known internal promoter architecture of φ29-family pRNA genes. Sequence-level similarity to φ29 pRNA was not detected by BLAST, which is unsurprising given that pRNAs from different φ29-family phages (φ29, B103, GA-1, SF5) share very little sequence identity while maintaining the same overall fold.

Methods

Sequence data was deposited in sequence read archive SRR33718591 and GenBank accession number PZ362296. For collection information, see PhagesDB/phages/MamaT.

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