# Complete genome sequence of phi29-like *Microbacterium foliorum* podovirus phage PineapplePizza

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**ABSTRACT** Bacteriophage PineapplePizza is a podovirus infecting *Microbacterium foliorum* NRRL B-24224. The genome is 16,662 bp long and contains 23 predicted protein-coding genes. Interestingly, PineapplePizza shows amino acid similarities to well-studied *Bacillus subtilis* phage phi29.

Bacteriophages infecting *Microbacterium* hosts are substantially diverse (1) and may be useful for bioremediation (2), *Microbacterium* genetics, and treatment of *Microbacterium* infections (1, 3). Of the sequenced phages infecting *Microbacterium foliorum*, four are singletons with no close relatives (4). Here we describe PineapplePizza, a singleton bacteriophage that infects *M. foliorum* and shares genomic features with *Bacillus subtilis* phage phi29.

PineapplePizza was isolated from soil in Amherst, Massachusetts, USA (global positioning system or GPS 42.3747N, 72.5196W) using standard methods (5).

Soil was suspended in peptone-yeast extract-calcium (PYCa) liquid medium, filtered through a 0.22-µm filter, and the filtrate plated in top agar with *Mi. foliorum* NRRL B-24224 and incubated at 30°C. PineapplePizza was purified with two rounds of plating and formed bullseye-type plaques with an average outer diameter of 2.3 mm after 48 h at 30°C. Negative-stain transmission electron microscopy showed PineapplePizza has podovirus morphology with capsids 40–43 nm wide and 42–48 nm long (Fig. 1, $n = 6$) and a tail length of 25 nm as measured using ImageJ v1.53r21 (6).

DNA was extracted from high titer lysates using a zinc chloride precipitation method (7), prepared for sequencing using the NEBNext Ultra II Kit (New England Biolabs, Ipswich, MA), and sequenced using an Illumina MiSeq instrument (v3 reagents) at the Pittsburgh Bacteriophage Institute (Pittsburgh, PA). Sequencing was performed to 9,214-fold coverage from 1,056,847 total single-end 150 bp reads. Assembly and quality control checks were performed with Newbler v2.9 and Consed v29.0, respectively (8, 9). The genome of PineapplePizza has 16,662 base pairs and a G + C content of 53.6%. No sequencing reads continued past the genome ends, and a 101-bp inverted repeat at the genome ends is consistent with covalently bound terminal proteins, as in phi29 (10). Whole-genome alignment with NCBI BLASTn (11) showed no significant nucleotide similarity to other *Microbacterium* phages, and PineapplePizza was classified as a singleton. The genome of PineapplePizza was autoannotated using Glimmer v3.02 (12) and GeneMark v2.5 (13), and then manually refined using Phamerator (14), DNA Master v5.23.6 (http://phagesdb.org/DNAMaster/), PECAAN, BLAST (11), and HHPred (15). No tRNA genes were identified by Aragorn v1.2.38 (16) or tRNAscan-SE v2.0 (17). All analyses were conducted using default settings.

PineapplePizza has 23 protein-encoding genes, of which 11 were assigned a function, and 15 were orphans (Table 1; Fig. 1). All but the first two genes are

## Microbacterium phage PineapplePizza
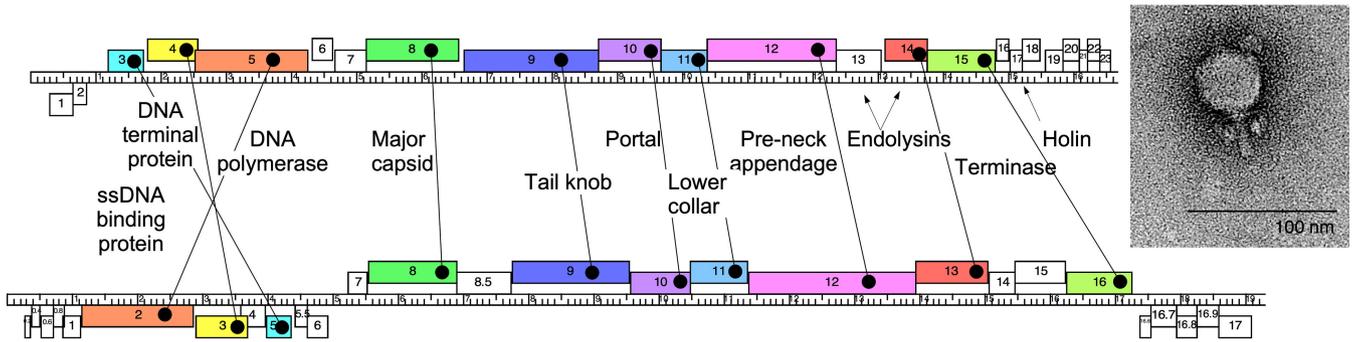


## Bacillus phage phi29

**FIG 1** Genome organization of *Microbacterium* phage PineapplePizza as compared to *Bacillus* phage phi29 (EU771092). The phage genome, as depicted by the ruler in the center of the figure, is displayed with genes represented by boxes above and below, reflecting rightward and leftward transcription, respectively. Gene boxes with color represent homologs to phi29, as defined in Table 1. The homologs are connected with black lines. Inset is of the phage particle using negative-stain transmission electron microscopy.

rightward-transcribed, consistent with architectural features of *Microbacterium* phages (Fig. 1) (1). PineapplePizza has 10 genes homologous to *Bacillus* phage phi29 as determined by HHPred (probability >97%, *E* value <6E−5, Table 1), including a ssDNA binding protein, DNA terminal protein, DNA polymerase, terminase, and six structural protein genes. A cryo-electron microscopy study of phi29 shows near-atomic detail

**TABLE 1** *Microbacterium* PineapplePizza gene functions and homologs

| Gene | Direction | Function | Phi29 homolog | Supporting data source hits[a] | *E* value | ID/SIM[b] |
|------|-----------|----------|---------------|----------------------------------|-----------|-----------|
| 1 | R | Hypothetical protein | – | NA | NA | NA |
| 2 | R | Hypothetical protein | – | NA | NA | NA |
| 3 | F | ssDNA binding protein | phi29_5 | PF17427.3 | 1e−38 | 13/28 |
| 4 | F | DNA terminal protein | phi29_3 | PF05435.14 | 2e−34 | 19/37 |
| 5 | F | DNA polymerase | phi29_2 | 2PY5_B | 2e−55 | 37/53 |
| 6 | F | Hypothetical protein | – | NA | NA | NA |
| 7 | F | Hypothetical protein | – | NA | NA | NA |
| 8 | F | Major capsid protein | phi29_8 | 6QZ0_7I | 9E−75 | 24/45 |
| 9 | F | Tail knob protein | phi29_9 | 5FB4_A | 1E−101 | 20/36 |
| 10 | F | Portal protein | phi29_10 | PF05352.15 | 2E−61 | 25/43 |
| 11 | F | Lower collar protein | phi29_11 | 6QZ9_0J | 2E−53 | 25/40 |
| 12 | F | Pre-neck appendage protein | phi29_12 | 3GQ8_A | 9E−14 | 17/29 |
| 13 | F | Endolysin, protease M15 domain | – | NA | NA | NA |
| 14 | F | Endolysin, protease M23 domain and cell wall binding domain | phi29_13 | P15132 | 6E−5 | 15/30 |
| 15 | F | Terminase | phi29_16 | P11014 | 6E−57 | 30/49 |
| 16 | F | Hypothetical protein | – | NA | NA | NA |
| 17 | F | Holin | – | NA | NA | NA |
| 18 | F | Hypothetical protein | – | NA | NA | NA |
| 19 | F | Hypothetical protein | – | NA | NA | NA |
| 20 | F | Hypothetical protein | – | NA | NA | NA |
| 21 | F | Hypothetical protein | – | NA | NA | NA |
| 22 | F | Hypothetical protein | – | NA | NA | NA |
| 23 | F | Hypothetical protein | – | NA | NA | NA |

[a]Supporting Data Source hits found at HHPred (15) with Pfam database v35, PDB_mmCIF70_14_Apr, and UniProt-SwissProt_viral70_3_Nov_2021.
[b]Needleman-Wunch global alignment percentage identity/similarity (NCBI: gap open/extend penalties = 11/1).
[c]– indicates that no phi29 homolog was detected.
[d]NA indicates not applicable.

of the entire bacteriophage particle (18) allowing for speculation that PineapplePizza has a similar structure. The tail knob, lower collar, and pre-neck appendage proteins found in phi29-family phages have not previously been identified in phages infecting *Microbacterium* hosts.

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## DATA AVAILABILITY

PineapplePizza is available at GenBank with Accession No. ON724010 and Sequence Read Archive (SRA) No. SRX14483237.

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