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# 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.

**KEYWORDS** bacteriophages

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- $\mu$ m 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

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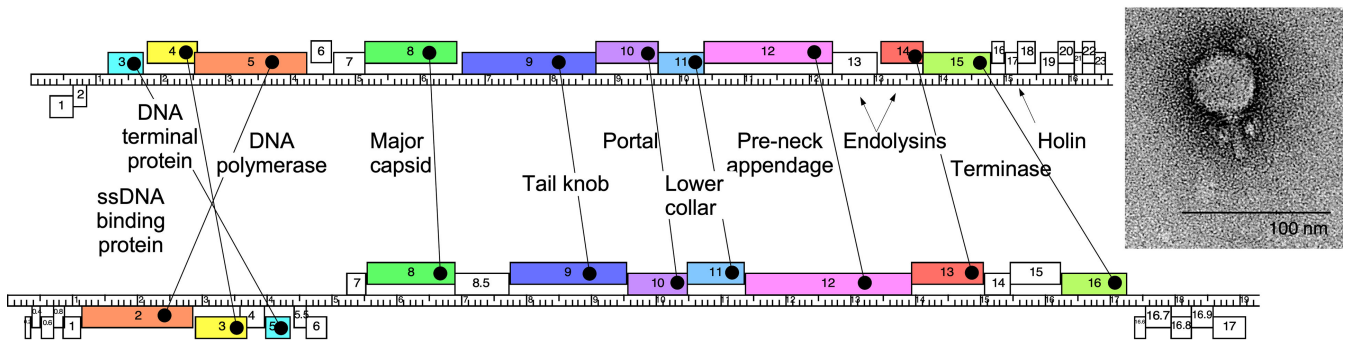
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## Microbacterium phage PineapplePizza



## 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 <sup>a</sup>	$E$ value	ID/SIM <sup>b</sup>
1	R	Hypothetical protein	–	NA	NA	NA
2	R	Hypothetical protein	–	NA	NA	NA
3	F	ssDNA binding protein	phi29_5	<a href="#">PF17427.3</a>	1e-38	13/28
4	F	DNA terminal protein	phi29_3	<a href="#">PF05435.14</a>	2e-34	19/37
5	F	DNA polymerase	phi29_2	<a href="#">2PY5_B</a>	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	<a href="#">6QZ0_7I</a>	9E-75	24/45
9	F	Tail knob protein	phi29_9	<a href="#">5FB4_A</a>	1E-101	20/36
10	F	Portal protein	phi29_10	<a href="#">PF05352.15</a>	2E-61	25/43
11	F	Lower collar protein	phi29_11	<a href="#">6QZ9_OJ</a>	2E-53	25/40
12	F	Pre-neck appendage protein	phi29_12	<a href="#">3GQ8_A</a>	9E-14	17/29
13	F	Endolysin, protease M15 domain	–	NA	NA	NA
		Endolysin, protease M23 domain and cell wall				
14	F	binding domain	phi29_13	<a href="#">P15132</a>	6E-5	15/30
15	F	Terminase	phi29_16	<a href="#">P11014</a>	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

<sup>a</sup>Supporting Data Source hits found at HHPred (15) with Pfam database v35, PDB\_mmCIF70\_14\_Apr, and UniProt-SwissProt\_viral70\_3\_Nov\_2021.

<sup>b</sup>Needleman-Wunch global alignment percentage identity/similarity (NCBI: gap open/extend penalties = 11/1).

– indicates that no phi29 homolog was detected.

<sup>d</sup>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](https://www.ncbi.nlm.nih.gov/nuclseq/ON724010) and Sequence Read Archive (SRA) No. [SRX14483237](https://www.ncbi.nlm.nih.gov/sra/SRX14483237).

## REFERENCES

- Jacobs-Sera D, Abad LA, Alvey RM, Anders KR, Aull HG, Bhalla SS, Blumer LS, Bollivar DW, Bonilla JA, Butela KA, Coomans RJ, Cresawn SG, D'Elia T, Diaz A, Divens AM, Edgington NP, Frederick GD, Gainey MD, Garlena RA, Grant KW, Gurney SMR, Hendrickson HL, Hughes LE, Kenna MA, Klyczek KK, Kotturi H, Mavrich TN, McKinney AL, Merkhofer EC, Moberg Parker J, Molloy SD, Monti DL, Pape-Zambito DA, Pollenz RS, Pope WH, Reyna NS, Rinehart CA, Russell DA, Shaffer CD, Sivanathan V, Stoner TH, Stuke J, Sunnen CN, Tolsma SS, Tsourkas PK, Wallen JR, Ware VC, Warner MH, Washington JM, Westover KM, Whitefleet-Smith JL, Wiersma-Koch HI, Williams DC, Zack KM, Hatfull GF. 2020. Genomic diversity of bacteriophages infecting *Microbacterium* spp. PLOS ONE 15:e0234636. <https://doi.org/10.1371/journal.pone.0234636>
- Ali N, Dashti N, Khanafer M, Al-Awadhi H, Radwan S. 2020. Bioremediation of soils saturated with spilled crude oil. Sci Rep 10:1116. <https://doi.org/10.1038/s41598-019-57224-x>
- Amano J, Hase R, Otsuka Y, Tsuchimochi T, Noguchi Y, Igarashi S. 2019. Catheter-related bloodstream infection by *Microbacterium paraoxydans* in a pediatric patient with B-cell precursor acute lymphocytic leukemia: a case report and review of literature on *Microbacterium* bacteremia. J Infect Chemother 25:806–810. <https://doi.org/10.1016/j.jiac.2019.03.013>
- Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage database. Bioinformatics 33:784–786. <https://doi.org/10.1093/bioinformatics/btw711>
- Poxleitner M, Pope WH, Jacobs-Sera D, Sivanathan V, Hatfull GF. SEA-PHAGES phage discovery guide. HHMI SEA-PHAGES Phage Discov Guide.

6. Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089>
7. Santos MA. 1991. An improved method for the small scale preparation of bacteriophage DNA based on phage precipitation by zinc chloride. *Nucleic Acids Res* 19:5442–5442. <https://doi.org/10.1093/nar/19.19.5442>
8. Russell DA. 2018. Sequencing, assembling, and finishing complete bacteriophage genomes, p. 109–125. In Clokie, MRJ, Kropinski, AM, Lavigne, R (eds.), *Bacteriophages*. Springer New York, NY.
9. Gordon D, Green P. 2013. Consed: a graphical editor for next-generation sequencing. *Bioinformatics* 29:2936–2937. <https://doi.org/10.1093/bioinformatics/btt515>
10. Ito J. 1978. Bacteriophage phi29 terminal protein: its association with the 5' termini of the phi29 genome. *J Virol* 28:895–904. <https://doi.org/10.1128/JVI.28.3.895-904.1978>
11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2)
12. Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679. <https://doi.org/10.1093/bioinformatics/btm009>
13. Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. *Nucleic Acids Res* 33:W451–4. <https://doi.org/10.1093/nar/gki487>
14. Cresawn SG, Bogel M, Day N, Jacobs-Sera D, Hendrix RW, Hatfull GF. 2011. Phamerator: a bioinformatic tool for comparative bacteriophage genomics. *BMC Bioinformatics* 12:395. <https://doi.org/10.1186/1471-2105-12-395>
15. Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. *Nucleic Acids Res* 33:W244–8. <https://doi.org/10.1093/nar/gki408>
16. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 32:11–16. <https://doi.org/10.1093/nar/gkh152>
17. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 25:955–964. <https://doi.org/10.1093/nar/25.5.955>
18. Xu J, Wang D, Gui M, Xiang Y. 2019. Structural assembly of the tailed bacteriophage  $\Phi$ 29. *Nat Commun* 10:2366. <https://doi.org/10.1038/s41467-019-10272-3>