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Complete genome sequences of Cluster FE *Arthrobacter globiformis* phages Piku and Utopia

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Abstract

Phages Piku and Utopia were isolated from soil samples in Illinois, USA, on *A. globiformis* B-2979 and B-2880, respectively. Both phages have genomes encoding 22 genes, including an endolysin. The genomes are highly conserved, differing by only three genes, including genes involved in structural and replication functions, and one of unknown function. Based on gene content, both phages are assigned to cluster FE.

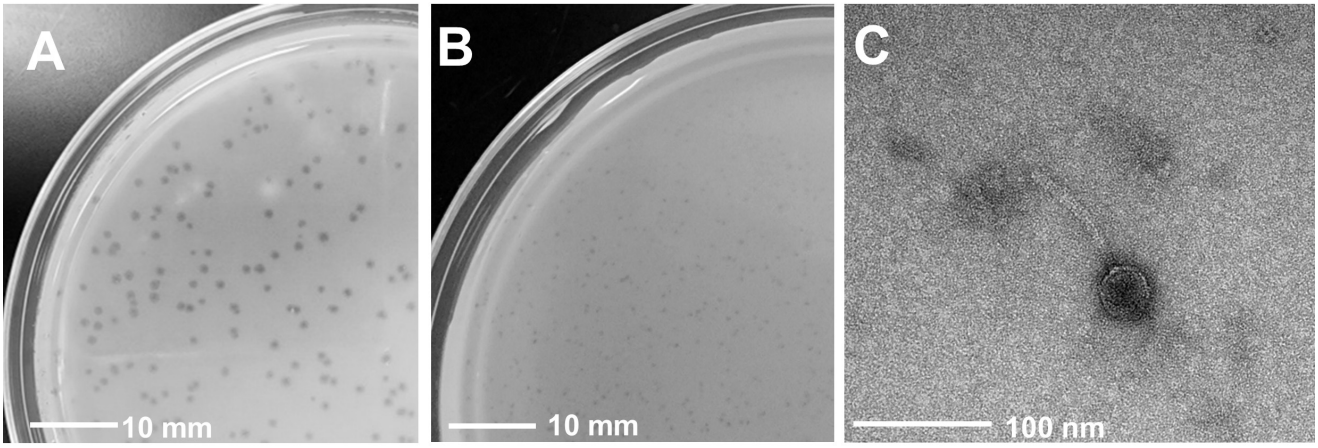

Figure 1. Plaque and viral morphologies:

Piku (A) had consistent 1 mm (n=3) plaques while Utopia (B) ranged from 0.5 to 1 mm (n=3) plaques. Transmission electron microscopy (TEM) shows Utopia with an icosahedral capsid and flexible tail suggestive of a siphovirus morphology. (C) Both phages contain a tape measure protein in their genome which suggests the presence of a tail. Scale bars for plaque images and TEM micrographs are 10 mm and 100 nm, respectively. Utopia TEM image generated at the University of Maryland, Baltimore County.

Description

Interest in using bacteriophages for clinical and agricultural applications, such as treating antibiotic-resistant infections or ensuring food safety, underscores the need to understand phage genomes and host interactions (Strathdee et al., 2023, Ranveer et al., 2024). To support this effort, the SEA-PHAGES program offers an effective platform for student researchers to contribute meaningfully to the expanding body of knowledge in phage biology (Heller et al., 2024).

Here, two bacteriophages, Piku and Utopia, were isolated using bacterial host *Arthrobacter globiformis* B-2979 and B-2880, respectively. Soil samples collected in Schaumburg and Oakbrook, Illinois (GPS coordinates in Table 1) were incubated with shaking in PYCa supplemented with cycloheximide at 22°C for 5 hours in the absence of the host bacterium (Zorawik et al., 2024). The filtered supernatants were plated in PYCa soft agar with *A. globiformis* B-2979 and *A. globiformis* B-2880, respectively and incubated for 48 hours at 22°C. Isolated phage plaques were purified a total of three times. Phages Piku and Utopia generated 0.5 – 1.0 mm (n=3) diameter plaques with clear centers and define edges (Figure 1A, B). Phage lysates were imaged by negative stain (1% uranyl acetate) transmission electron microscopy (TEM) revealing a siphovirus morphology for Utopia; micrographs for Piku were of insufficient resolution to determine its structure (Figure 1C).

DNA was extracted from high titer lysates using Promega Wizard DNA Cleanup kit, libraries were constructed using the NEB Next Ultra II FS kit, and samples were sequenced for Piku (Illumina MiSeq 1000) and Utopia (Illumina NextSeq 1000). Single-end 150-base raw reads for Piku were assembled using Newbler (Miller, et al., 2010) and genome termini were identified using Consed v29 (Gordon and Green, 2013). Single-end 100-base raw reads for Utopia were trimmed with cutadapt 4.7 and filtered with skewer 0.2.2 prior to assembly with Newbler V2.9, as previously described (Russell, 2018). Sequencing data and genome characteristics, including length, GC%, and number of genes, are reported in Table 1. Piku and Utopia were assigned to Cluster FE based on gene content similarity of at least 35% to phages in the database PhagesDB (Russell and Hatfull 2017; Pope et al. 2017).

Genome annotation was performed using DNA Master V5.23.6 (Jacobs-Sera et al., 2014) with gene predictions from Glimmer (Delcher et al., 2007), and further refined using Starterator (Pacey, 2016), GeneMark v2.5 (Besemer and Borodovsky, 2005), and Phamerator v596 (Cresawn et al., 2011). Protein sequences were analyzed for similarity, transmembrane domains, and functions using the Actinobacteriophage and NCBI non-redundant database (Altschul et al., 1990), TMHMM v2 (Hallgren et al., 2011), and HHpred v2.08 with selected databases PDB_mmCIF70, SCOPe70, UnitProt-SwissProt-viral70, SMART_v6.0 (Soding et al., 2005). The presence of tRNAs were examined using the programs Aragorn and tRNAscanSE (Lowe and Eddy, 1997, Laslett and Canback, 2004). All software programs were used with their default setting.

Using the Gene Content Similarity (GCS) tool in PhagesDB, phage Piku and Utopia share 90.9% of their phams (clusters of phage proteins with high amino acid similarity) (Cresawn et al. 2011, Russell and Hatfull, 2017). Piku and Utopia encode 22 predicted genes, of which 15 and 14 genes, respectively, were assigned a putative function. No tRNAs were identified, consistent with the other 12 phages assigned to Cluster FE (Kotturi et al., 2024). All genes in both genomes are transcribed unidirectionally.

The left arm of the genome contains structural genes, followed by a putative lysis cassette consisting of an endolysin and two genes with predicted transmembrane domains (two and one domains, respectively) that may encode a holin. Based on the absence of identifiable lysogeny-related genes, both phages are predicted to follow a lytic replication cycle. The remaining genes encode diverse functions, including helix-turn-helix DNA-binding domain proteins, DNA methyltransferases, HNH endonucleases, and seven to eight proteins of unknown function. Notably, Piku is the only phage in the cluster known to encode a putative DNA methyltransferase.

Nucleotide sequence accession numbers

Piku is available through NCBI GenBank Accession number PQ362670 and sequence read archive (SRA) Number SRR33718586.

Utopia is available through NCBI GenBank Accession number PV876972 and sequence read archive (SRA) Number SRR33718576.

Table 1: Soil collection site, average shotgun coverage, cluster assignment, and genome summary (size, ends, GC %, and number of genes).

Phage name	**Piku**	**Utopia**
Location site (GPS)	42.03 N, 88.10 W	41.83 N, 87.98 W
Average shotgun coverage	6511X	25899X
Genome size (bp)	15675 bp	15346 bp
Genome termini	3' single-stranded overhang 5’-CCACGGTCCCCGTCC-3’	3’ single-stranded overhang 5’-CCACGGTCCCCGTCC-3’
GC content %	64.2%	64.7%
Number of Genes	22	22
Cluster	FE	FE

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References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ 1990. Basic local alignment search tool. *Journal of Molecular Biology* 215: 403–410. PubMed ID: 2231712

Bendele M, Cobb I, Cresawn S. *Subclusters*. Observable. Accessed 2025 Sep 19.

Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Res 33(Web Server issue): W451-4. PubMed ID: 15980510

Cobb I, Cooper K, Bendele M, Fisher R, Jones Z, Shifflett Z, Cresawn S. *Pham matrices.* Observable. Accessed 2025 Sep 19.

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. PubMed ID: 21991981

Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27(23): 4636-41. PubMed ID: 10556321

Gordon D, Green P. 2013. Consed: a graphical editor for next-generation sequencing. Bioinformatics 29(22): 2936-7. PubMed ID: 23995391

Hallgren J, Tsirigos KD, Pedersen MD, Almagro Armenteros JJ, Marcatili P, Nielsen H, Krogh A, Winther O. 2022. DeepTMHMM predicts alpha and beta transmembrane proteins using deep neural networks. : 10.1101/2022.04.08.487609. DOI: 10.1101/2022.04.08.487609

Heller DM, Sivanathan V, Asai DJ, Hatfull GF. 2024. SEA-PHAGES and SEA-GENES: Advancing Virology and Science Education. Annu Rev Virol 11(1): 1-20. PubMed ID: 38684129

Jacobs Sera D, Pope W, Russell D, Bowman C, Cresawn S, Hatfull G. 2014. Annotation and bioinformatic analysis of bacteriophage genomes: a user guide to DNA Master. *Software guide*. PhagesDB.org.

Kotturi H, Adair T, Perez Morales T, Monti D. 2024. Cluster FE annotation report. HHMI Science Education Alliance (SEA) Faculty Group, QUBES Educational Resources. DOI: 10.25334/XX87-E226

Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32(1): 11-6. PubMed ID: 14704338

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res 25(5): 955-64. PubMed ID: 9023104

Miller JR, Koren S, Sutton G. 2010. Assembly algorithms for next-generation sequencing data. Genomics 95(6): 315-27. PubMed ID: 20211242

Pacey M. 2016. Starterator: a guide to phage gene start site identification. *Software manual*. University of Pittsburgh, PA.

Pope WH, Mavrich TN, Garlena RA, Guerrero-Bustamante CA, Jacobs-Sera D, Montgomery MT, et al., Hatfull GF. 2017. Bacteriophages of Gordonia spp. Display a Spectrum of Diversity and Genetic Relationships. mBio 8(4): 10.1128/mBio.01069-17. PubMed ID: 28811342

Ranveer SA, Dasriya V, Ahmad MF, Dhillon HS, Samtiya M, Shama E, et al., Puniya AK. 2024. Positive and negative aspects of bacteriophages and their immense role in the food chain. NPJ Sci Food 8(1): 1. PubMed ID: 38172179

Russell D, Hatfull G. 2017. PhagesDB: the actinobacteriophage database. PubMed ID: 28365761

Russell DA. 2018. Sequencing, Assembling, and Finishing Complete Bacteriophage Genomes. Methods Mol Biol 1681: 109-125. PubMed ID: 29134591

Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Res 33(Web Server issue): W244-8. PubMed ID: 15980461

Strathdee SA, Hatfull GF, Mutalik VK, Schooley RT. 2023. Phage therapy: From biological mechanisms to future directions. Cell 186(1): 17-31. PubMed ID: 36608652

Zorawik M, Jacobs-Sera D, Freise AC, SEA-PHAGES, Reddi K. 2024. Isolation of Bacteriophages on Actinobacteria Hosts. Methods Mol Biol 2793: 273-298. PubMed ID: 38526736

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