

## Genome Sequence of *Arthrobacter* Phage KNG13

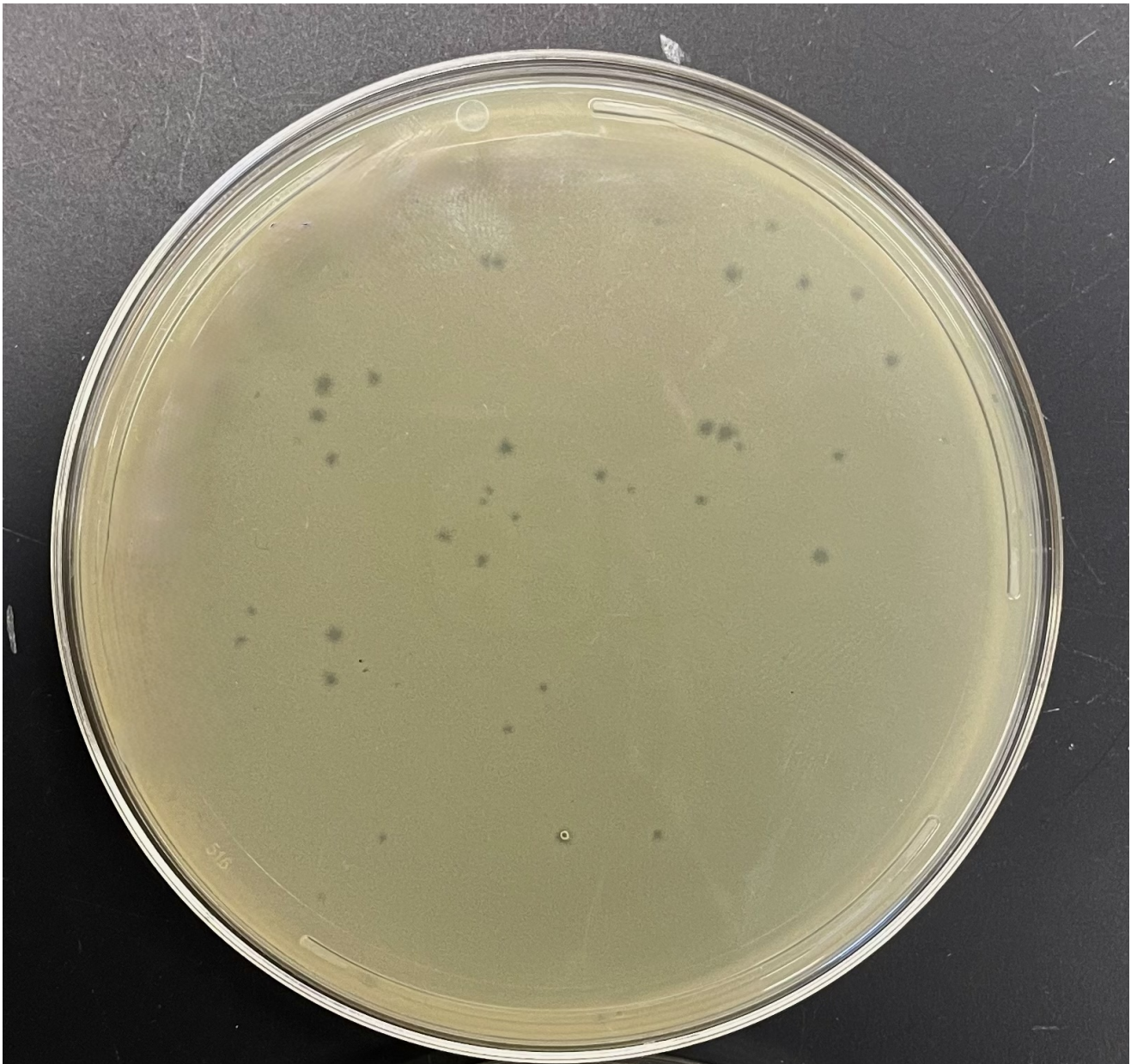
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### Abstract

Bacteriophage KNG13, a siphovirus, was isolated in San Antonio, Texas, USA, using *Arthrobacter globiformis* B-2979. KNG13 has a genome length of 15,768-bp and is assigned to actinobacteriophage cluster FE based on gene content.



**Figure 1. Plaque morphology of KNG13:**

Clear, circular plaques of KNG13 on a 100 mm PYCa agar plate using an *Arthrobacter globiformis* lawn.

## Description

Bacteriophages are incredibly abundant and diverse, with multitude of applications ranging from phage therapy to biotechnology (Dedrick et al., 2019; Naureen et al., 2020; Rogovski et al., 2021). Here, we describe the isolation and characterization of a novel bacteriophage, KNG13.

Phage KNG13 was extracted from mildly damp soil collected on a trail in San Antonio, Texas, USA (29.663765 N, 98.421426 W) when the ambient temperature was approximately 27°C. The soil sample was processed using standard protocols (Zorawik et al., 2024). Briefly, approximately 7 cm<sup>3</sup> of soil sample was suspended in 10 ml PYCa (peptone, yeast extract, calcium chloride, and dextrose) liquid medium and agitated in a shaking incubator for 2 hours. The suspension was then spun (2,000 x g for 10 minutes) and the supernatant filtered using a syringe filter (0.2-micron pore size). The filtrate was subsequently plated in PYCa top agar with *Arthrobacter globiformis* B-2979 and plates incubated at 30 °C for 48 hours. Phage KNG13, which forms plaques that are clear and approximately 1.5 mm in diameter (Figure 1), was purified by two rounds of selecting plaques and plating.

A lysate for KNG13 was prepared (2.8 x 10<sup>9</sup> PFU/mL) and used to extract DNA with the Promega Wizard DNA kit. Phage DNA was then prepared for sequencing with the NEB Ultra FS kit and sequenced on an Illumina NextSeq 1000 (XLEAP-P1 kit), yielding 1,345,255 single-end 100 base reads. Raw sequencing reads were trimmed using cutadapt v4.7 (using the option: `-nextseq-trim 30`) and subsequently filtered with skewer v0.2.2 (using the options: `-q 20 -Q 30 -n -l 50`) prior to assembly. The genome was assembled using Newbler v.29 (Miller et al., 2010) and checked for completeness using Consed v2.9 (Gordon et al., 1998), resulting in an assembled genome of 15,768 base pairs with 7,815-fold coverage. The genome consisted of 67.6 % GC content, with 3' single-stranded overhangs of a 5'CCACGTATAACCGTCC.

The genome was annotated with DNA Master v5.23.6 (cobamide2.bio.pitt.edu) and PECAAN v20250130 (discover.kbrinsgd.org) (Rinehart et al., 2016), using Glimmer v3.02 (Delcher et al. 2007 ) and GeneMark v2.5p (Besemer and Borodovsky 2005) to predict genes and Starterator v605 (<http://phages.wustl.edu/starterator/>) to refine start coordinates. Putative gene functions were assigned using Phamerator v593 (Cresawn et al., 2011), HHPred (Söding 2005) searches against the the PDB\_mmCIF70, Pfam- v.36, NCBI Conserved Domains databases and NCBI Blastp v2.16.0 (Altschul et al. 1990) searches against the Actinobacteriophage and NCBI non-redundant databases. DeepTMHMM v1.0.42 (Hallgren et al. 2022) was used to identify transmembrane domains and tRNAscanSE 2.0 (Lowe & Eddy 1997) to identify tRNAs. All software were used with default settings.

Based on gene content similarity of at least 35% to phages in the Actinobacteriophage database, KNG13 was assigned to actinobacteriophage cluster FE (Pope et al., 2017; Russell & Hatfull, 2016). A total of 26 putative genes were identified, of which 13 could be assigned putative functions. These include several functions related to virion structure and assembly, an endolysin, two proteins with DNA-binding domains, one HNH endonuclease, and a RepA-like replication initiator. Consistent with cluster FE phages, the gene encoding the endolysin is adjacent to two genes that encode transmembrane domains, one of which is assigned as a holin in a subset of phages. The RepA-like replication initiator protein of KNG13 is grouped in a protein family with members only encoded in 4 other FE phages, to date. No lysogeny-related functions could be identified, suggesting KNG13 is unlikely to establish lysogeny.

## Nucleotide sequence accession numbers

KNG13 is available at GenBank with Accession No. PV876960 and Sequence Read Archive (SRA) No. [SRX31241838](https://www.ncbi.nlm.nih.gov/sra/SRX31241838).

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