microPublication BIOLOGY

11/7/2024 - *Open Access*

Genome Sequence of *Arthrobacter globiformis B-2979* Phage *JanetJ*

Arib Ahsan[1*], Ruby S Crosthwait[1*], Josephine J Crosthwait[1*], Srilekha Davuluri[2*], Omoye N Ehimare[1*], Yihao Fan[1*], Tiarra Nikitha Joseph Philomen Raju[3*], Jamie Kim[1*], Alexander Y Lee[1*], Nicholas K Odani[3*], Mugil V Shanmugam[3*], Anmol Singhal[3*], Alana J Snyder[1,4*], Jessica W Sy[5*], Grace Y Wang[1*], George W Zhou[1*], Christa T Bancroft[1§*]

[1]Department of Biological Sciences, University of Southern California, Los Angeles, California, United States

[2]Department of Sociology, University of Southern California, Los Angeles, California, United States

[3]Department of Quantitative and Computational Biology, University of Southern California, Los Angeles, California, United States

[4]Department of Chemistry, University of Southern California, Los Angeles, California, United States

[5]Department of Gerontology, University of Southern California, Los Angeles, California, United States

[§]To whom correspondence should be addressed: cbancrof@usc.edu

[*]These authors contributed equally.

Abstract

Phage JanetJ was isolated on *Arthrobacter globiformis B-2979* and has siphovirus morphology. JanetJ's genome consists of 36,986 base pairs, encoding 52 putative protein-coding genes. JanetJ adds to the small number of previously isolated cluster FO phages, none of which encode identifiable immunity repressor or integrase functions, with the exception of phage Maja.

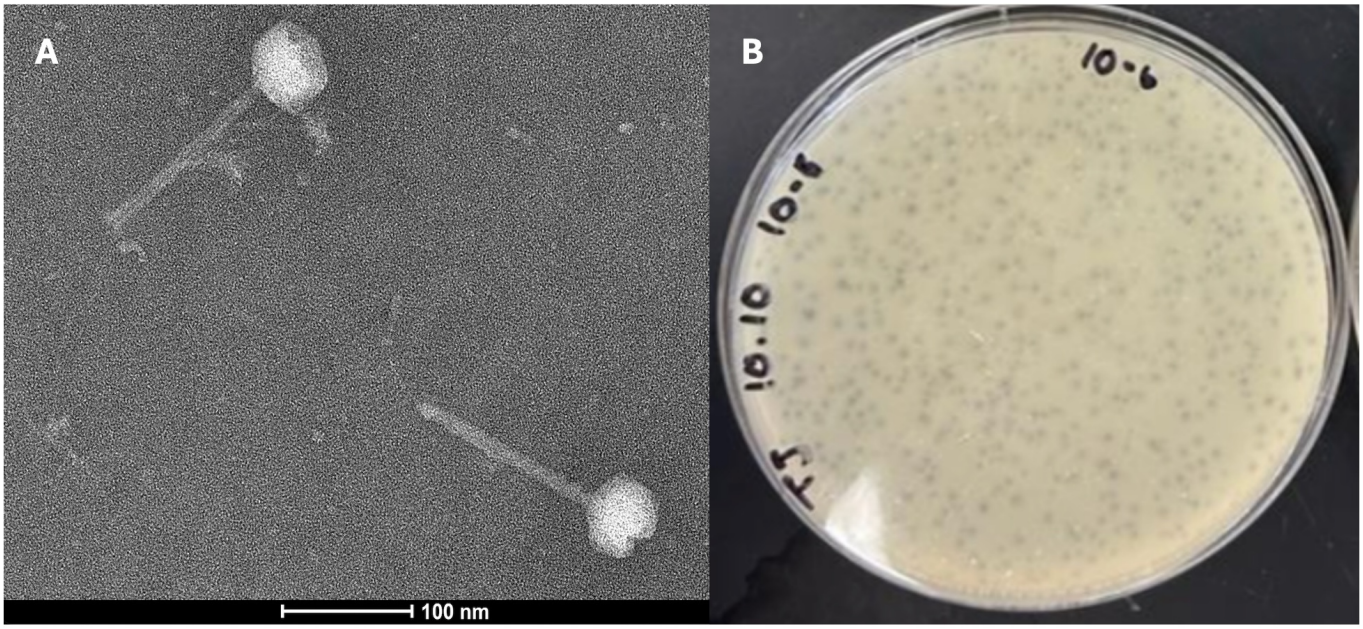

Figure 1. :

A) Nano-W negative stain (https://www.nanoprobes.com/products/Negative-Stains.html#nano-w) transmission electron micrograph (Talos F200CG2, 200KeV) (top left) and B) plaques for JanetJ. Scale bar is 100 nm.

Description

As some of the simplest yet most diverse biological entities in the world, bacteriophages have gained relevance today in both clinical and ecological spheres of research (Hatfull 2022). We aimed to discover phages that infect Arthrobacter, a genus that is host to a growing collection of phages and will permit population-level comparative genomics (Klyczek et al., 2017). Phage JanetJ was isolated from a moist soil sample collected at the University of Southern California (34.019432 N, 118.285946 W) using standard procedures (Poxleitner et al., 2018). Briefly, the soil sample was washed in peptone-yeast extract-calcium (PYCa) medium, the wash was filtered (0.22mm), the filtrate was inoculated with *Arthrobacter globiformis B-2979* and incubated with shaking at 30˚C for 48 h. The culture was refiltered, diluted and plated in soft agar containing *Arthrobacter*

globiformis B-2979. After 24 h at 30˚C, JanetJ produced slightly cloudy, round, and uniform plaques, with a diameter of 1.35-1.5 mm (n=7) (Figure 1). The phage were purified with 4 rounds of plating before being imaged by negative-stain (methylamine tungstate; Nano-W) transmission electron microscopy. JanetJ has siphovirus morphology, possessing a tail 145-147 nm in length and capsid of 54-56 nm in diameter (n=3) (Figure 1).

Double-stranded DNA was purified using the Promega Wizard DNA cleanup kit, prepared for sequencing using the NEB Ultra II kit, and sequenced on an Illumina MiSeq (v3 reagents). Sequencing reads were assembled using Newbler v2.9 and checked for accuracy and genomic termini using Consed v29 (Gordon and Green 2013), as described previously (Russell and Hatfull 2018) yielding a 36,986 bp genome with 3' single-stranded overhang (Table 1).

The genome sequence was automatically annotated using DNAMaster v5.23.6 (cobamide2.bio.pitt.edu) embedded with GeneMark v2.0 (Besemer and Borodovsky 2005) and Glimmer v3.02 (Delcher et al., 2007). Following auto-annotation, Starterator (http://phages.wustl.edu/starterator/) was used to refine start sites. JanetJ encodes 52 putative protein-coding genes. No tRNAs were identified by Aragorn v1.2.38 (Laslett and Canback 2004) and tRNAscan-SE 2.0 (Lowe and Eddy 1997). Default parameters were used for all software. Based on gene-content similarity of at least 35% to phages in the Actinobacteriophage database (Russell and Hatfull 2016), JanetJ is assigned to phage cluster FO. HHPred (databases: PDB mmCIF70, Pfam-A, and NCBI Conserved Domain databases) (Söding et al., 2005), NCBI BLAST (databases: nonredundant and Actinobacteriophage) (Altschul et al., 1990), and Phamerator (database Actino_Draft) (Cresawn et al., 2011) were used to deduce the putative functions of proteins encoded by open reading frames. Of note, no immunity repressor or integrase functions could be identified, suggesting JanetJ is unlikely to establish lysogeny. This is in contrast to one other cluster FO phage, Maja, which encodes a putative immunity repressor and two integrase genes, though no experimental data for lysogeny has been reported for Maja. While Maja and JanetJ have 35% gene content similarity, the majority of gene conservation is within the first third of the genome encoding structure and assembly genes, whereas the remaining two-thirds, including the region encoding the lysogeny functions in Maja, is poorly conserved with JanetJ and other cluster FO phages. The genes in this latter region of cluster FO phages include those associated with DNA metabolism and many with unknown functions. Across all cluster FO phages, most of the genes are transcribed unidirectionally, with the exception of a handful of genes in each genome that are transcribed in the opposite direction, including those encoding the immunity repressor and one integrase in Maja and two putative helix-turn-helix DNA binding domain proteins in JanetJ.

Table 1. Sequencing Data and Genome Characteristics for JanetJ

Number of Sequencing Reads	426,273
Length of Sequencing Reads	150-base single-end
Coverage of Sequencing Reads	1728x
Genome Length (bp)	36,986
GC%	68.10%
Genome End Types	3' sticky overhangs (5' - TTCGCCTGGTA - 3')
Cluster Assignment	FO

Nucleotide Sequence Accession and Read Numbers

JanetJ is available at GenBank accession PP978789 and Sequence Read Archive (SRA) accession SRX24123888

Acknowledgements:

This research was made possible by the HHMI SEA-PHAGES program, Dr. Graham Hatfull's group at the University of Pittsburgh, and the University of Southern California's Department of Biological Sciences. We would like to give a special thanks to Dr. Raffaella Ghittoni and Dr. Christa Bancroft for their teaching, support, and mentorship throughout this research

endeavor, as well as Dr. Bancroft's guidance in producing this manuscript. Images presented in this article were acquired at the Core Center of Excellence in Nano Imaging at the University of Southern California.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403-410. DOI: 10.1016/S0022-2836(05)80360-2

Besemer J, Borodovsky M. 2005. GeneMark: web software for gene finding in prokaryotes, eukaryotes and viruses. Nucleic Acids Research 33: W451-W454. DOI: 10.1093/nar/gki487

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: 10.1186/1471-2105-12-395. DOI: 10.1186/1471-2105-12-395

Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics 23: 673-679. DOI: 10.1093/bioinformatics/btm009

Gordon D, Green P. 2013. Consed: a graphical editor for next-generation sequencing. Bioinformatics 29:2936–2937

Hatfull GF. 2022. Mycobacteriophages: From Petri dish to patient. PLOS Pathogens 18: e1010602. DOI: 10.1371/journal.ppat.1010602

Klyczek KK, Bonilla JA, Jacobs-Sera D, Adair TL, Afram P, Allen KG, et al., Hatfull. 2017. Tales of diversity: Genomic and morphological characteristics of forty-six Arthrobacter phages. PLOS ONE 12: e0180517. DOI: 10.1371/journal.pone.0180517

Laslett D. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Research 32: 11-16. DOI: 10.1093/nar/gkh152

Lowe TM, Eddy SR. 1997. tRNAscan-SE: A Program for Improved Detection of Transfer RNA Genes in Genomic Sequence. Nucleic Acids Research 25: 0955-964. DOI: 10.1093/nar/25.5.0955

Poxleitner M, Pope W, Jacobs-Sera D, Sivanathan V, Hatfull GF. 2018. HHMI SEA-PHAGES Phage Discovery Guide. https://seaphagesphagediscoveryguide. helpdocsonline.com/home

Russell DA, Hatfull GF. 2016. PhagesDB: the actinobacteriophage database. Bioinformatics 33: 784-786. DOI: 10.1093/bioinformatics/btw711

Russell DA, Hatfull GF. 2018. Sequencing, assembling, and finishing complete bacteriophage genomes. Methods Mol Biol 1681:109–125.

Söding J, Biegert A, Lupas AN. 2005. The HHpred interactive server for protein homology detection and structure prediction. Nucleic Acids Research 33: W244-W248. DOI: 10.1093/nar/gki408

Funding:

Funding for the SEA-PHAGES lab was provided by the Biological Sciences Department at the University of Southern California

Author Contributions: Arib Ahsan: data curation, formal analysis, investigation, validation, writing - original draft. Ruby S Crosthwait: data curation, formal analysis, investigation, validation, writing - original draft. Josephine J Crosthwait: data curation, formal analysis, investigation, validation, writing - original draft. Srilekha Davuluri: data curation, formal analysis, investigation, supervision, software, validation, writing - original draft. Omoye N Ehimare: data curation, formal analysis, investigation, validation, writing - original draft. Yihao Fan: data curation, formal analysis, investigation, validation, writing - original draft. Tiarra Nikitha Joseph Philomen Raju: data curation, formal analysis, investigation, validation, writing - original draft. Jamie Kim: data curation, formal analysis, investigation, validation, writing - original draft. Alexander Y Lee: data curation, formal analysis, investigation, validation, writing - original draft. Nicholas K Odani: data curation, investigation, validation, writing - original draft, formal analysis. Mugil V Shanmugam: data curation, formal analysis, investigation, validation, writing - original draft. Anmol Singhal: data curation, formal analysis, investigation, validation, writing - original draft. Alana J Snyder: data curation, investigation, validation, writing - original draft, formal analysis. Jessica W Sy: investigation, data curation, validation, writing - original draft, formal analysis. Grace Y Wang: investigation, data curation, formal analysis, validation, writing - original draft. George W Zhou: data curation, formal analysis, validation, writing - original draft, investigation. Christa T Bancroft: data curation, formal analysis, project administration, supervision, writing - review editing, writing - original draft.

11/7/2024 - *Open Access*

Reviewed By: Anonymous

History: Received September 13, 2024 **Revision Received** November 7, 2024 **Accepted** November 7, 2024 **Published Online** November 7, 2024 **Indexed** November 21, 2024

Citation: Ahsan, A; Crosthwait, RS; Crosthwait, JJ; Davuluri, S; Ehimare, ON; Fan, Y; et al.; Bancroft, CT (2024). Genome Sequence of *Arthrobacter globiformis B-2979* Phage *JanetJ*. microPublication Biology. 10.17912/micropub.biology.001351