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Characterization of the Complete Genome of *Arthrobacter globiformis* Cluster FF Bacteriophage QuinnAvery

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Abstract

Phage QuinnAvery produces turbid plaques in *Arthrobacter globiformis* B-2979 lawns, displays a siphovirus morphology, and is clustered with FF actinobacteriophages. The 43,177 bp genome of QuinnAvery is predicted to contain a tRNA-Arg(TCT) gene and 69 protein-coding genes. Homologs of a subset of QuinnAvery's predicted genes have putative roles in viral structure, particle assembly, host cell lysis, and lysogeny. QuinnAvery's genome is predicted to contain two

tyrosine integrases, similar to many other cluster FF phages. However, the area immediately surrounding the integration cassette in QuinnAvery is distinct from all other annotated members of cluster FF.

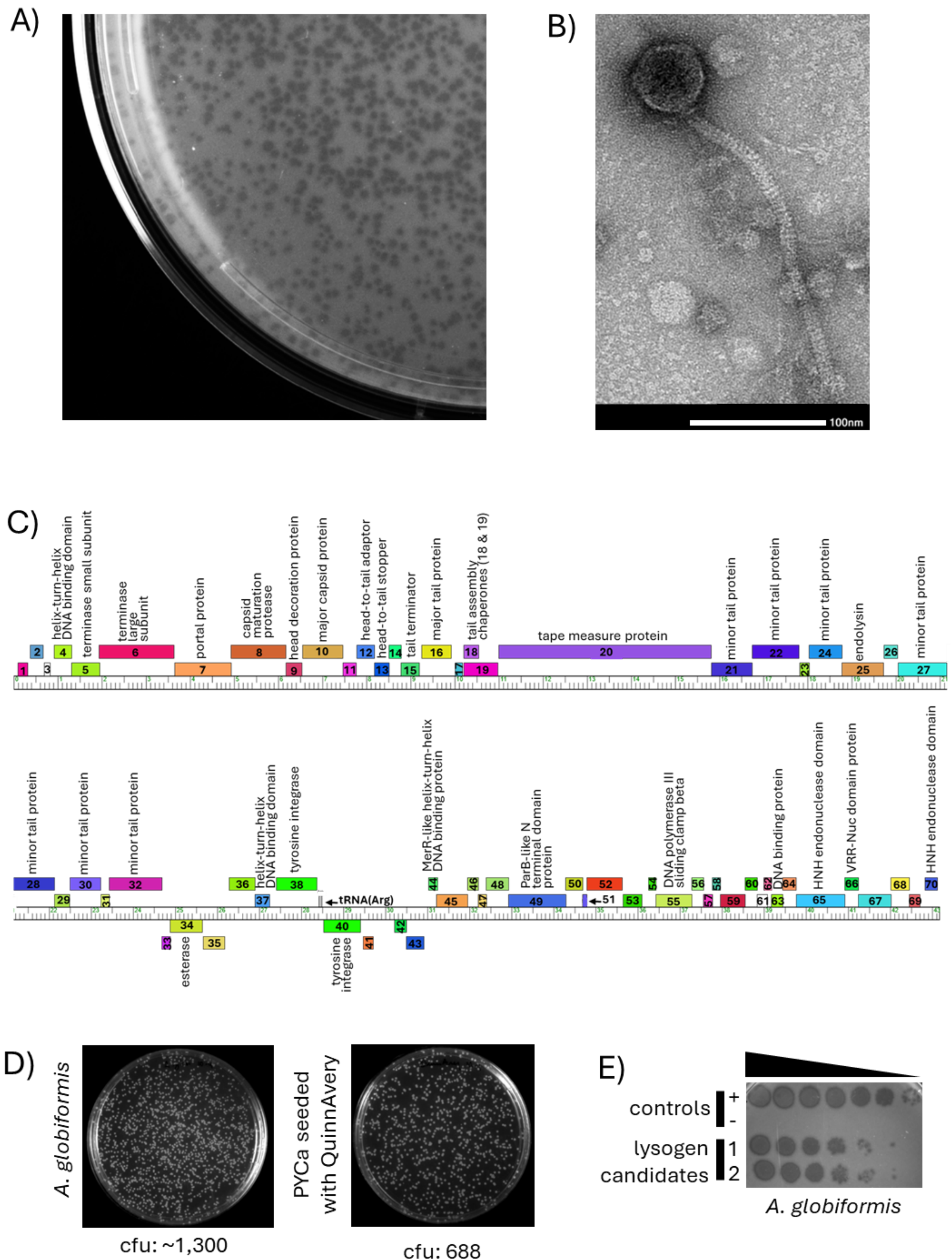

Figure 1. Phage QuinnAvery morphological and genomic characteristics:

(A) QuinnAvery produces turbid plaques in an *A. globiformis* B-2979 lawn embedded in an 0.4% PYCa agar overlay after incubation at 30 ^{o}C for 3 days. Plaques average 0.73 millimeters (+/- 0.16; n=40; image is magnified 2-fold). (B) QuinnAvery virion particle morphology was elucidated by transmission electron microscopy of particles stained with 1% wt/vol uranyl acetate. Images were captured using a Hitachi HT7800 at 80k magnification. (C) Phamerator map depicting predicted size, position, and function of 70 genes in the QuinnAvery genome. (D) Lysogens harboring QuinnAvery prophage elements are generated in approximately 53% of host cells. An equal sample of *A. globiformis* B-2979 was spread on a PYCa plate (left) and a PYCa plate that had been seeded with 10^8 QuinnAvery plaque forming units (right) and incubated for 3 days at 30 ^{o}C. Plates show an efficiency of plating of approximately 53%. (E) Supernatant was collected from liquid cultures of two QuinnAvery lysogen candidates (1 and 2), serially diluted, and plated on a lawn of *A. globiformis* B-2979 and incubated at 30°C for 1 day to assay for phage release. Controls (+ = QuinnAvery lysate; - = phage buffer) were also serially diluted and plated in parallel.

Description

The global diversity of bacteriophages is exceptionally large and diverse (Hendrix et al., 1999; Pedulla et al., 2003; Suttle et al., 2005). Continuous identification and characterization of novel phages has significantly enhanced our understanding of viral function and evolution (Hendrix et al., 1999; Mavrich and Hatfull, 2017; Hatfull, 2022). To contribute to ongoing efforts to explore bacteriophage diversity, we report here the discovery and initial characterization of QuinnAvery, an *Arthrobacter globiformis* phage.

Phage QuinnAvery was discovered utilizing SEA-PHAGES protocols (Zorawik et al., 2024). Briefly, an environmental soil sample was collected in Linthicum Heights, Maryland, USA (39.2015 N, 76.68484 W) in June 2024. Soil was agitated in peptone-yeast extract-calcium (PYCa) liquid medium in the presence of *A. globiformis* B-2979 at 30°C for 24 hours. Following centrifugation, the supernatant was passed through a 0.22 mm polycarbonate filter to remove residual soil particulates and bacterial cells. A fraction of the collected filtrate was mixed with 0.4% top agar and *A. globiformis* and overlaid onto a solid PYCa plate. Turbid plaques measuring 0.73 millimeters (+/- 0.16; n=40) were present following incubation at 30°C for 1 day (Figure 1A). Well-isolated plaques were utilized for three rounds of purification prior to generation of a high titer liquid lysate. Transmission electron microscopy was performed on a sample of lysate that had been negatively stained with 1% uranyl acetate. This analysis revealed that phage QuinnAvery displays siphovirus morphology with a capsid diameter of approximately 51.5 nm (+/- 0.25 nm) in diameter and a tail length of approximately 258 nm (+/- 1 nm) (n=2; Figure 1B).

Genomic DNA was extracted from a sample of high titer lysate utilizing the Promega Wizard DNA Clean-Up Kit, sequencing libraries were created with the Illumina XLEAP-P1 kit, and DNA sequencing was performed on an Illumina NextSeq 1000 to approximately 5,933-fold coverage. Approximately 4,000,000 raw single-end 150-bp reads were trimmed with cutadapt 4.7 (using the option: –nextseq-trim 30) and filtered with skewer 0.2.2 (using the options: -q 20 -Q 30 -n -l 50) prior to assembly and finished using Unicycler 0.5.1 (Wick et al., 2017) and Consed v.29 (Gordon et al., 2013) with default parameters. These analyses determined that the genome of Quinn Avery is 43,177 bp in length, is composed of 64.9% GC nucleotide pairs, and terminates in a 3’ single-stranded overhang (5’-TCCGCCGCGTGA-3’). Using the Gene Content tool on PhagesDB (Russell and Hatfull, 2017), QuinnAvery shares between 71% to 79% gene content similarity with annotated cluster FF phages, including Elesar, Cole, Guasanita, Nandita, Popper, Ryan, and Zaheer. This degree of similarity exceeds the threshold value of 35% gene content similarity (Pope et al., 2017b) necessary for QuinnAvery to be assigned to actinobacteriophage Cluster FF.

Initial auto-annotation was performed using Glimmer v3.02 (Delcher et al., 1999) and GeneMark v2.5 (Besemer & Borodovsky, 2005) and manually refined using Phamerator v580 (Cresawn et al., 2011), DNA Master v5.23.6 (Pope and Jacobs-Sera, 2018), Starterator v587 (github.com/SEA-PHAGES/starterator), and PECAAN (discover.kbrinsgd.org). Functional assignments were evaluated using BLASTp v 2.2.26 (using NCBI nonredundant database and the database at phagesdb.org; Altschul et al., 1990) and HHpred (using PDB_mmCIF70, PfamA, and NCBI Conserved Domain databases; Söding et al., 2005). A single tRNA^{ARG} gene was identified by Aragorn v1.2.38 (Laslett & Canback, 2004) and tRNAscan-SE v2.0 (Lowe & Chan, 2016). The presence of transmembrane helices was evaluated with Deep TMMHM v1.0.42 (Hallgren et al., 2022). Default parameters were utilized for all programs other than DNA Master, which was set according to instructions in the SEA-PHAGES bioinformatics guide (Pope, Jacobs-Sera et al., 2017).

The finalized annotation includes a total of 69 predicted protein-coding genes, of which 39 were assigned putative functions (Figure 1C). The first thirty-two predicted genes in the left arm of the chromosome are oriented in the forward direction and contain an abundance of genes predicted to encode structural and assembly proteins. Multiple genes with predicted roles in nucleic acid processing and metabolism are located in the final 27 forward-oriented genes at the right end of the genome. Genes with predicted roles in lysogeny are centrally located in an area of the genome where gene orientation switches twice from reverse to forward. Of the eight annotated cluster FF phages, all but Elesar contain two predicted tyrosine integrases. The identification of multiple candidate integrases in a phage genome is rare (Hatfull et al.,

2010; Klyczek et al., 2017), though it has been observed previously in a small number of *Arthrobacter* and *Gordonia* phages (Pope, Mavrich and Hatfull, 2017). Although lysogen candidates harboring QuinnAvery prophage have been isolated (Figures 1D and 1E), additional studies are necessary to confirm whether both integrases (genes 38 and 40), which share only 44% amino acid similarity, are functional (Wise and Sivanathan, 2025).

All previously annotated cluster FF phages with two predicted integrases contain at least one predicted gene between the integrases in addition to the tRNA^{ARG}; the identity and orientation of these genes is variable. QuinnAvery is unusual in that there are no predicted protein-coding genes between its predicted integrases; these genes are separated only by the tRNA^{ARG} gene. QuinnAvery is also unique among cluster FF phages in that it contains two forward genes positioned upstream of the first predicted integrase; gene 36 has no recognizable functional domains, but gene 37 is predicted to contain a helix-turn-helix motif. Elucidation of the functions of these proteins may yield interesting insights into the complexities of establishing and maintaining lysogeny.

Nucleotide sequence accession numbers

QuinnAvery is available at GenBank with Accession No. PQ844483 and Sequence Read Archive (SRA) No. SRX27124803.

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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. 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. DOI: 10.1093/bioinformatics/btt515

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

Hatfull GF, Jacobs-Sera D, Lawrence JG, Pope WH, Russell DA, Ko CC, et al., Hendrix. 2010. Comparative Genomic Analysis of 60 Mycobacteriophage Genomes: Genome Clustering, Gene Acquisition, and Gene Size. Journal of Molecular Biology 397: 119-143. DOI: 10.1016/j.jmb.2010.01.011

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

Hendrix RW, Smith MCM, Burns RN, Ford ME, Hatfull GF. 1999. Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage. Proceedings of the National Academy of Sciences 96: 2192-2197. DOI: 10.1073/pnas.96.5.2192

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, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Research 44: W54-W57. DOI: 10.1093/nar/gkw413

Mavrich TN, Hatfull GF. 2017. Bacteriophage evolution differs by host, lifestyle and genome. Nature Microbiology 2: 10.1038/nmicrobiol.2017.112. DOI: 10.1038/nmicrobiol.2017.112

Pedulla ML, Ford ME, Houtz JM, Karthikeyan T, Wadsworth C, Lewis JA, et al., Hatfull. 2003. Origins of Highly Mosaic Mycobacteriophage Genomes. Cell 113: 171-182. DOI: 10.1016/s0092-8674(03)00233-2

Pope WH, Jacobs-Sera D. 2017. Annotation of Bacteriophage Genome Sequences Using DNA Master: An Overview. Methods in Molecular Biology,Bacteriophages : 217-229. DOI: 10.1007/978-1-4939-7343-9_16

Pope WH, Jacobs-Sera DJ, Russell DA, Cresawn SG, Hatfull GF. 2017a. HHMI SEA-PHAGES Bioinformatics Guide. https://seaphagesbioinformatics.helpdocsonline.com/home

Pope WH, Mavrich TN, Garlena RA, Guerrero-Bustamante CA, Jacobs-Sera D, Montgomery MT, et al., Hatfull. 2017. Bacteriophages of *Gordonia* spp. Display a Spectrum of Diversity and Genetic Relationships. mBio 8: 10.1128/mbio.01069-17. DOI: 10.1128/mBio.01069-17

Rinehart CA, Gaffney B, Wood, JD, Smith J. 2016. PECAAN, a Phage Evidence Collection and Annotation Network. https://discover.kbrinsgd.org/login

Russell DA, Hatfull GF. 2017. PhagesDB: the actinobacteriophage database. Bioinformatics 33(5): 784-786. PubMed ID: 28365761

Soding 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

Suttle CA. 2005. Viruses in the sea. Nature 437: 356-361. DOI: 10.1038/nature04160

Wise B, Sivanathan V. Successful Lysogen Formation for Cluster FF Phage, QuinnAvery. HHMI Science Education Alliance (SEA) Faculty Group, (Version 2.0). QUBES Educational Resources. 2025; doi:10.25334/QSC0-ET79 DOI: 10.25334/QSC0-ET79

Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS Computational Biology 13: e1005595. DOI: 10.1371/journal.pcbi.1005595

Zorawik M, Jacobs-Sera D, Freise AC, SEA-PHAGES, Reddi K. 2024. Isolation of Bacteriophages on Actinobacteria Hosts. Methods in Molecular Biology,Phage Engineering and Analysis : 273-298. DOI: 10.1007/978-1-0716-3798-2_17

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