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GENOME SEQUENCES

Complete Genome Sequence of Broad-Host-Range *Staphylococcus aureus* Myophage ESa1

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Roshan D'Souza and Andrey A. Filippov contributed equally to this work. Roshan D'Souza performed the genomic assembly and bioinformatics analysis. Andrey A. Filippov isolated and characterized the bacteriophage and isolated genomic DNA. Both authors wrote the manuscript. Author order was determined alphabetically by surname.

ABSTRACT A potentially therapeutic Twort-like myophage, Esa1, with specificity toward *Staphylococcus aureus* was isolated from lake water. We report the complete genome sequence of ESa1, assembled using both MinION and Illumina MiSeq reads, consisting of 153,106 bp, with 30.3% GC content, 253 protein coding sequences, 4 tRNAs, and 10,437-bp direct terminal repeats.

Staphylococcus aureus is an important human pathogen prone to methicillin resistance that limits treatment options (1, 2). Bacteriophages (phages) have been effective against *S. aureus* infections in animals and humans (3). Here, we report the complete genome sequence of phage ESa1, isolated from lake water in Frederick, MD, using *S. aureus* strain MRSN 8383 for enrichment. Eight milliliters of water sterilized by filtration through a 0.22-μm membrane was mixed with 2 ml of 5× heart infusion broth (HIB; Becton, Dickinson and Co., Franklin Lakes, NJ) and 50 μl of bacterial culture grown in 1× HIB for 16 h at 37°C with shaking at 200 rpm. The mix was incubated for 16 h at 37°C in a shaking incubator at 200 rpm. Bacterial debris was removed by centrifugation, and the supernatant was filter sterilized. Serial 10-fold dilutions in sodium chloride-magnesium sulfate (SM) buffer (Teknova, Hollister, CA) were spotted onto double-layer (0.7% top/1.5% bottom) HIB agar containing 100 μl of overnight *S. aureus* MRSN 8383 culture in the top semisolid agar layer. The next day, confluent lysis spots and single phage plaques were observed. A well-isolated phage plaque was picked up with a micropipette tip and suspended in SM buffer, and the phage suspension was filter sterilized.

Phage ESa1 was purified by three rounds of single plaque isolation. The final plaque suspension was used for phage propagation by adding it to 150 ml of an early-exponential-phase culture of *S. aureus* MRSN 8383 in HIB at a multiplicity of infection of 0.01 and incubating it for 6 h at 37°C with shaking at 200 rpm. Lysis was completed by adding chloroform to a final concentration of 5% (vol/vol). Bacterial debris was removed by centrifugation for 15 min at 5,000 × *g*; phage was concentrated by centrifugation for 4 h at 13,000 × *g* and resuspended in SM buffer in 1/100 of the original volume (4). Phage genomic DNA was isolated by removal of host RNA and DNA using RNase A and DNase, deproteinization using Proteinase K, and SDS treatment at 56°C, purification by phenol-chloroform extraction, overnight precipitation with ethanol at −20°C, and centrifugation and resuspension in nuclease-free water as previously described (4).

The complete genome of ESa1 was determined using MinION and Illumina MiSeq sequencing. Illumina sequencing libraries were prepared using a NEBNext Ultra II library

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preparation kit and sequenced with a 300-cycle SP reagent kit on a NovaSeq 6000 using the Xp workflow (Illumina, San Diego, CA, USA), generating 40,811 paired-end reads with 12 million bases. Default parameters were used for all software unless otherwise specified. Quality control of reads was done with FastQC v0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and trimming was performed using Trimmomatic v0.32 (5) with the following settings: ILLUMINACLIP, TruSeq3-PE-2.fa:2:30:10; LEADING, 3; TRAILING, 3; SLIDINGWINDOW, 4:24; and MINLEN, 60. MinION sequencing libraries were prepared using the rapid barcoding kit (SQK-RBK004) and sequenced using a MinION R9.4 flow cell (Nanopore, Oxford, UK), which generated 694 reads with an N_{50} value of 14,765 bp. Base calling was performed live during sequencing with MinKNOW v3.4.8 in fast mode with Guppy v3.0.7 (Nanopore) integrated. These reads were demultiplexed and trimmed using Porechop v0.2.3 (6). The Unicycler v0.4.7 (7) pipeline was used for hybrid *de novo* assembly of Illumina and MinION reads. The average depths of read coverage for the final assembly, calculated using Geneious v10.0.9, were 12.5× and 82.22× for MinION and Illumina reads, respectively. Assembly errors were corrected using Pilon v1.22 (8). Annotation was performed using the RAST server (9). tRNA genes were predicted using ARAGORN v1.2.38 (10) and tRNAscan-SE v2.0 (11).

The ESa1 genome is 153,106 bp long with a GC content of 30.3%, 253 protein-coding sequences, and 4 tRNAs. PhageTerm (12) detected direct terminal repeats of 10,437 bp. A Web-based MegaBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search indicated that ESa1 has 96.7% and 96.2% nucleotide identities over the whole genome to *S. aureus* phages JD007 (GenBank accession number JX878671) and Maine (GenBank accession number MN045228), respectively. Similar to these previously identified phages, the genome sequence of ESa1 contains long terminal repeats and lacks GATC sequences and thus Sau3AI, BamHI, PvuI, and DpnI sites (13–15). ESa1 is a Twort-like myophage and appears to belong to the family *Herelleviridae*, subfamily *Torovirinae*, and genus *Kayvirus* (https://talk.ictvonline.org/taxonomy). Genome analysis revealed the presence of one intron-like sequence (pol-I2) in a DNA polymerase gene. Whole-genome alignments and RepeatFinder (http://www.cbcb.umd.edu/software/RepeatFinder/) did not reveal a locus similar to a putative host range determinant, complex repeat region 2 of Sb-1, another Twort-like myophage (4). Like other Twort-like viruses (15), ESa1 is a broad-host-range lytic phage and a promising therapeutic candidate.

Data availability. The phage ESa1 genome sequence was submitted to GenBank under accession number MT554104. The associated BioProject, SRA, and BioSample accession numbers are PRJNA632562, SRP261805, and SAMN14912224, respectively.

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REFERENCES

1. Tong SYC, Davis JS, Eichenberger E, Holland TL, Fowler VG, Jr. 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev 28:603–661. https://doi.org/10.1128/CMR.00134-14.
2. Diekema DJ, Pfaller MA, Shortridge D, Zervos M, Jones RN. 2019. Twenty-year trends in antimicrobial susceptibilities among *Staphylococcus aureus* from the SENTRY Antimicrobial Surveillance Program. Open Forum Infect Dis 6:S47–S53. https://doi.org/10.1093/ofid/ofy270.
3. Azam AH, Tanji Y. 2019. Peculiarities of *Staphylococcus aureus* phages and their possible application in phage therapy. Appl Microbiol Biotechnol 103:4279–4289. https://doi.org/10.1007/s00253-019-09810-2.
4. Sergueev KV, Filippov AA, Farlow J, Su W, Kvachadze L, Balarjishvili N,

Kutateladze M, Nikolich MP. 2019. Correlation of host range expansion of therapeutic bacteriophage Sb-1 with allele state at a hypervariable repeat locus. Appl Environ Microbiol 85:e01209-19. https://doi.org/10.1128/AEM.01209-19.
5. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
6. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genom 3:e000132. https://doi.org/10.1099/mgen.0.000132.
7. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
8. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal.pone.0112963.
9. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
10. 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.
11. 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.
12. Garneau JR, Depardieu F, Fortier L-C, Bikard D, Monot M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. Sci Rep 7:8292. https://doi.org/10.1038/s41598-017-07910-5.
13. Cui Z, Song Z, Wang Y, Zeng L, Shen W, Wang Z, Li Q, He P, Qin J, Guo X. 2012. Complete genome sequence of wide-host-range *Staphylococcus aureus* phage JD007. J Virol 86:13880–13881. https://doi.org/10.1128/JVI.02728-12.
14. Moreland R, Korn A, Newkirk H, Liu M, Gill JJ, Cahill J, Ramsey J. 2019. Complete genome sequence of *Staphylococcus aureus* myophage Maine. Microbiol Resour Announc 8:e01050-19. https://doi.org/10.1128/MRA.01050-19.
15. Łobocka M, Hejnowicz MS, Dąbrowski K, Gozdek A, Kosakowski J, Witkowska M, Ulatowska MI, Weber-Dąbrowska B, Kwiatek M, Parasion S, Gawor J, Kosowska H, Głowacka A. 2012. Genomics of staphylococcal Twort-like phages—potential therapeutics of the post-antibiotic era. Adv Virus Res 83:143–216. https://doi.org/10.1016/B978-0-12-394438-2.00005-0.