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GENOME SEQUENCES

Complete Genome Sequence of *Staphylococcus aureus* Siphophage Sebago

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ABSTRACT Here, we introduce the genome of Sebago, a 43,878-bp siphophage that infects *Staphylococcus aureus*. Sebago carries 70 proteins and is most closely related to StauST398, a *Phietavirus*.

Staphylococcus aureus is a Gram-positive, nonsporulating, commensal microbe that affects both humans and animals (1). While typically present in the normal flora of human skin and nares, various strains of *S. aureus*, such as methicillin-resistant *S. aureus* (MRSA), have obtained antibiotic resistance and become leading causes of nosocomial infections (2). To combat the antibiotic resistance in *S. aureus*, alternative treatments using bacteriophage are in development (3). Here we present the complete genome of Sebago, a siphophage infecting *S. aureus*.

Sebago was isolated from a filtered (0.2-μm) Minnesota swine barn environmental swab eluate via an overnight enrichment against *S. aureus* strain PD17 (Texas swine nasal isolate; *spa* type t034) (4). The phage and host propagation were carried out aerobically at 30°C in tryptic soy broth (TSB) medium (Difco) using the soft-agar overlay method (5). The genomic DNA for Sebago was purified with the Promega Wizard DNA cleanup kit according to the modification in the shotgun library preparation protocol given by Summer (6), with an additional 10 mM EDTA (pH 8.0) and 100 μg/ml proteinase K treatment for 30 min at 50°C after polyethylene glycol (PEG) precipitation (to eliminate heat-stable staphylococcal nucleases). To generate 250-bp paired libraries, we used an Illumina TruSeq Nano low-throughput kit (6). The Illumina MiSeq index using v2 500-cycle chemistry yielded 372,373 total reads. FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for quality control. FastX-Toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/) was used for trimming. The Sebago contig was assembled using SPAdes v3.5.0 with 87-fold coverage (7). The genome was confirmed as complete via PCR (forward primer, 5′-CTGCCAAAGTCTG TAGCAATAAC-3′; reverse primer, 5′-TTGCTTACTGGCGACTTCTC-3′) and Sanger sequencing of the product. Annotation was done in Web Apollo, first calling genes with GLIMMER v3.0, MetaGeneAnnotator v1.0, and ARAGORN v2.36 for tRNAs (8–11). Terminators predicted as rho-independent were identified with TransTermHP (12). Functional prediction made use of evidence from TMHMM v2.0, InterProScan v5.22, and BLAST against the NCBI nonredundant and UniProtKB Swiss-Prot/TrEMBL databases (13–16). The annotation tools were run in the Galaxy instance hosted using default parameters by the Center for Phage Technology at Texas A&M University (https://cpt.tamu.edu/galaxy-pub/) (17). To ascertain the phage morphology, Sebago samples were negatively stained with 2% (wt/vol) uranyl acetate and viewed by transmission electron microscopy at the Texas A&M Microscopy and Imaging Center (18).

Sebago has a 43,878-bp genome with a 94.5% coding density. There are 70 protein-coding genes, with 37 functional predictions made, and no tRNA genes. Sebago's 35.3% G+C content is similar to the ~33% of its host *S. aureus* (19).

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PhageTerm predicts a headful packaging mechanism for this phage (20). In progressiveMauve and BLASTp comparisons, Sebago has 77.4% overall nucleotide identity and 54 genes similar to those of another *S. aureus* siphophage, StauST398 (GenBank accession no. JQ973847) (21, 22). StauST398, a *Phietavirus*, has a genome size, total number of genes, and G+C content similar to those of Sebago.

Interestingly, in the Sebago genome, the putative tape measure protein (GenBank accession no. QBQ72253) is adjacent to the predicted tail assembly chaperone (QBQ72255) and its frameshifted product (QBQ72254), similar to the phage lambda G/GT chaperone system (23).

Data availability. The genome sequence and associated data for phage Sebago were deposited under GenBank accession no. MK618716, BioProject accession no. PRJNA222858, SRA accession no. SRR8869228, and BioSample accession no. SAMN11360406.

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REFERENCES

1. Thomer L, Schneewind O, Missiakas D. 2016. Pathogenesis of Staphylococcus aureus bloodstream infections. Annu Rev Pathol 11:343–364. https://doi.org/10.1146/annurev-pathol-012615-044351.
2. 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.
3. Grunenwald CM, Bennett MR, Skaar EP. 2018. Nonconventional therapeutics against Staphylococcus aureus. Microbiol Spectr 6. https://doi.org/10.1128/microbiolspec.GPP3-0047-2018.
4. Sun J, Yang M, Sreevatsan S, Davies PR. 2015. Prevalence and characterization of Staphylococcus aureus in growing pigs in the USA. PLoS One 10:e0143670. https://doi.org/10.1371/journal.pone.0143670.
5. Adams MH. 1956. Bacteriophages. Interscience Publishers, Inc., New York, NY.
6. Summer EJ. 2009. Preparation of a phage DNA fragment library for whole genome shotgun sequencing. Methods Mol Biol 502:27–46. 2nd ed. https://doi.org/10.1007/978-1-60327-565-1_4.
7. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
8. Lee E, Helt GA, Reese JT, Munoz-Torres MC, Childers CP, Buels RM, Stein L, Holmes IH, Elsik CG, Lewis SE. 2013. Web Apollo: a Web-based genomic annotation editing platform. Genome Biol 14:R93. https://doi.org/10.1186/gb-2013-14-8-r93.
9. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27:4636–4641. https://doi.org/10.1093/nar/27.23.4636.
10. Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction in anonymous prokaryotic and phage genomes. DNA Res 15:387–396. https://doi.org/10.1093/dnares/dsn027.
11. 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.
12. Kingsford CL, Ayanbule K, Salzberg SL. 2007. Rapid, accurate, computational discovery of Rho-independent transcription terminators illuminates their relationship to DNA uptake. Genome Biol 8:R22. https://doi.org/10.1186/gb-2007-8-2-r22.
13. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580. https://doi.org/10.1006/jmbi.2000.4315.
14. Jones P, Binns D, Chang H-Y, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong S-Y, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics 30:1236–1240. https://doi.org/10.1093/bioinformatics/btu031.
15. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.
16. The UniProt Consortium. 2018. UniProt: the universal protein knowledgebase. Nucleic Acids Res 46:2699. https://doi.org/10.1093/nar/gky092.
17. Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Grüning BA, Guerler A, Hillman-Jackson J, Hiltemann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544. https://doi.org/10.1093/nar/gky379.
18. Valentine RC, Shapiro BM, Stadtman ER. 1968. Regulation of glutamine synthetase. XII. Electron microscopy of the enzyme from Escherichia coli. Biochemistry 7:2143–2152. https://doi.org/10.1021/bi00846a017.
19. Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K. 2008. Genome sequence of Staphylococcus aureus strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. J Bacteriol 190:300–310. https://doi.org/10.1128/JB.01000-07.
20. 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.
21. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
22. Hernandez D, van der Mee-Marquet N, Kluytmans J, Donnio P-Y, Quentin R, Corvaglia A-R, François P. 2013. Whole-genome sequences of Staphylococcus aureus ST398 strains of animal origin. Genome Announc 1:255. https://doi.org/10.1128/genomeA.00689-13.
23. Xu J, Hendrix RW, Duda RL. 2014. Chaperone-protein interactions that mediate assembly of the bacteriophage lambda tail to the correct length. J Mol Biol 426:1004–1018. https://doi.org/10.1016/j.jmb.2013.06.040.