

Coding-Complete Genome Sequence of Staphylococcus aureus Podophage Portland

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ABSTRACT Staphylococcus aureus bacteria, especially the multidrug resistance strains, are responsible for a wide range of clinical infections. Here, we announce the genome sequence of S. aureus podophage Portland, which is closely related to a group of phi29-like S. aureus podophages, including phages phi44AHJD and phiP68. The exact genome sequence ends of phage Portland were not determined and may be obscured by terminal proteins.

*S*taphylococcus aureus is a major human pathogen responsible for a wide range of clinical infections, including pneumonia, bacteremia, hospital-acquired wound infections, and medical device-associated infections [\(1\)](#page-1-0). The use of different types of antibiotics over the years has led to the emergence of methicillin-resistant S. aureus (MRSA) strains that are often resistant to other classes of antibiotics [\(2\)](#page-1-1). Given the limited antibiotic treatment options, S. aureus phages may have clinical promise as therapeutic agents for the treatment of S. aureus infections [\(3\)](#page-1-2).

Phage Portland was isolated from environmental samples collected from a swine barn in Kansas in 2015 against S. aureus strain NRS253. Host bacteria were cultured on tryptic soy broth or agar (Difco) at 30°C with aeration. Phages were cultured and propagated using the soft agar overlay method [\(4\)](#page-1-3). Portland was identified as a podophage using negative-stain transmission electron microscopy performed at the Texas A&M University Microscopy and Imaging Center, as described previously [\(5\)](#page-1-4). Phage genomic DNA was prepared using a modified Promega Wizard DNA cleanup kit protocol [\(5\)](#page-1-4). Pooled indexed DNA libraries were prepared using the Illumina TruSeq Nano low-throughput (LT) kit, and the sequence was obtained from the Illumina MiSeq platform using the MiSeq V2 500-cycle reagent kit, following the manufacturer's instructions, producing 597,167 paired-end 250-bp reads for the index containing the phage Portland genome. FastQC 0.11.5 [\(https://www.bioinformatics.babraham.ac](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) [.uk/projects/fastqc/\)](https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to quality control the reads. The reads were trimmed with the FASTX-Toolkit 0.0.14 [\(http://hannonlab.cshl.edu/fastx_toolkit/download.html\)](http://hannonlab.cshl.edu/fastx_toolkit/download.html) before being assembled using SPAdes 3.5.0 [\(6\)](#page-1-5). Glimmer 3.0 [\(7\)](#page-1-6) and MetaGene-Annotator 1.0 [\(8\)](#page-1-7) were used to predict protein-coding genes, with manual verification, and tRNA genes were predicted using ARAGORN 2.36 [\(9\)](#page-1-8). Rho-independent termination sites were identified via TransTermHP [\(http://transterm.cbcb.umd.edu/\)](http://transterm.cbcb.umd.edu/). Sequence similarity searches were done using BLASTp 2.2.28 [\(10\)](#page-1-9), with a maximum expectation cutoff of 0.001 against the NCBI nonredundant (nr), UniProt Swiss-Prot [\(11\)](#page-1-10), and TrEMBL databases. InterProScan 5.15-54.0 [\(12\)](#page-1-11), LipoP [\(13\)](#page-1-12), and TMHMM v2.0 [\(14\)](#page-1-13) were used to predict protein functions. All analyses were conducted at default settings via the CPT Galaxy [\(15\)](#page-1-14) and Web Apollo [\(16\)](#page-1-15) interfaces [\(https://cpt.tamu.edu/galaxy-pub\)](https://cpt.tamu.edu/galaxy-pub).

Phage Portland was assembled at 78.6-fold coverage into a contig of 17,711 bp. Portland is closely related to a group of phi29-like S. aureus podophages [\(17,](#page-1-16) [18\)](#page-1-17), within which phages phi44AHJD and phiP68 have been characterized [\(19\)](#page-1-18). Portland possesses a low G-C content (29.6%) similar to those of its host (32.7%) [\(20\)](#page-2-0) and to this group

Citation Bonasera RM, Korn A, Newkirk H, O'Leary C, Gill J, Liu M. 2019. Coding-complete genome sequence of Staphylococcus aureus podophage Portland. Microbiol Resour Announc 8:e01337-19. [https://doi.org/10.1128/](https://doi.org/10.1128/MRA.01337-19) [MRA.01337-19.](https://doi.org/10.1128/MRA.01337-19)

Editor Simon Roux, DOE Joint Genome Institute

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Received 23 October 2019 **Accepted** 28 October 2019 **Published** 21 November 2019 of phages [\(19\)](#page-1-18). As determined using BLASTn against the NCBI nucleotide database, phage Portland shares 89% and 83% nucleotide similarity with S. aureus phages phiP68 (GenBank accession number [AF513033\)](https://www.ncbi.nlm.nih.gov/nuccore/AF513033) and phi44AHJD (GenBank accession number [AF513032\)](https://www.ncbi.nlm.nih.gov/nuccore/AF513032), respectively. All 19 predicted proteins in Portland share homology with phage phiP68 (BLASTp; E value, $\leq 10^{-29}$). Direct amplification using primers facing off the Portland genome ends failed to generate a PCR product, consistent with the presence of covalently linked terminal proteins described in this phage group [\(19,](#page-1-18) [21,](#page-2-1) [22\)](#page-2-2). The Portland genome reported in this study is likely missing short sequences from each contig end. Experiments were not conducted to determine the extreme terminal sequences of the Portland chromosome, which may be obscured by terminal proteins.

Data availability. The genome sequence of phage Portland was submitted to GenBank as accession number [MN098325.](https://www.ncbi.nlm.nih.gov/nuccore/MN098325) The associated BioProject, SRA, and BioSample accession numbers are [PRJNA222858,](https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA222858) [SRR8761742,](https://www.ncbi.nlm.nih.gov/sra/?term=SRR8761742) and [SAMN11191518,](https://www.ncbi.nlm.nih.gov/biosample/SAMN11191518) respectively.

ACKNOWLEDGMENTS

This work was supported by funding from the National Science Foundation (awards EF-0949351 and DBI-1565146) and by the National Pork Board (project number 16-143). Additional support came from the Center for Phage Technology (CPT), an Initial University Multidisciplinary Research Initiative supported by Texas A&M University and Texas AgriLife, and the Department of Biochemistry and Biophysics at Texas A&M University.

The S. aureus strain HT 20020354 (NR-46046) was provided by the Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) for distribution by BEI Resources, NIAID, NIH. We thank Raghavendra Amachawadi at Kansas State University for collecting samples. We are grateful for the advice and support of the CPT staff and the Texas A&M University Microscopy and Imaging Center.

This announcement was prepared in partial fulfillment of the requirements for BICH464 Phage Genomics, an undergraduate course at Texas A&M University.

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.](https://doi.org/10.1128/CMR.00134-14)
- 2. Livermore DM. 2000. Antibiotic resistance in staphylococci. Int J Antimicrob Agents 16:S3–S10. [https://doi.org/10.1016/S0924-8579\(00\)00299-5.](https://doi.org/10.1016/S0924-8579(00)00299-5)
- 3. Chhibber S, Shukla A, Kaur S. 2017. Transfersomal phage cocktail is an effective treatment against methicillin-resistant Staphylococcus aureusmediated skin and soft tissue infections. Antimicrob Agents Chemother 61:e02146-16. [https://doi.org/10.1128/AAC.02146-16.](https://doi.org/10.1128/AAC.02146-16)
- 4. Adams MK. 1959. Bacteriophages. Interscience Publishers, Inc., New York, NY.
- 5. Gill JJ, Berry JD, Russell WK, Lessor L, Escobar-Garcia DA, Hernandez D, Kane A, Keene J, Maddox M, Martin R, Mohan S, Thorn AM, Russell DH, Young R. 2012. The Caulobacter crescentus phage phiCbK: genomics of a canonical phage. BMC Genomics 13:542. [https://doi.org/10.1186/1471](https://doi.org/10.1186/1471-2164-13-542) [-2164-13-542.](https://doi.org/10.1186/1471-2164-13-542)
- 6. 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.](https://doi.org/10.1089/cmb.2012.0021)
- 7. 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.](https://doi.org/10.1093/nar/27.23.4636)
- 8. 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.](https://doi.org/10.1093/dnares/dsn027)
- 9. 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.](https://doi.org/10.1093/nar/gkh152)
- 10. 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.](https://doi.org/10.1186/1471-2105-10-421)

- 11. The UniProt Consortium. 2018. UniProt: the universal protein knowledgebase. Nucleic Acids Res 46:2699. [https://doi.org/10.1093/nar/](https://doi.org/10.1093/nar/gky092) [gky092.](https://doi.org/10.1093/nar/gky092)
- 12. 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.](https://doi.org/10.1093/bioinformatics/btu031)
- 13. Juncker AS, Willenbrock H, Von Heijne G, Brunak S, Nielsen H, Krogh A. 2003. Prediction of lipoprotein signal peptides in Gram-negative bacteria. Protein Sci 12:1652–1662. [https://doi.org/10.1110/ps.0303703.](https://doi.org/10.1110/ps.0303703)
- 14. 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](https://doi.org/10.1006/jmbi.2000.4315) [.1006/jmbi.2000.4315.](https://doi.org/10.1006/jmbi.2000.4315)
- 15. Cock PJ, Grüning BA, Paszkiewicz K, Pritchard L. 2013. Galaxy tools and workflows for sequence analysis with applications in molecular plant pathology. PeerJ 1:e167. [https://doi.org/10.7717/peerj.167.](https://doi.org/10.7717/peerj.167)
- 16. 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](https://doi.org/10.1186/gb-2013-14-8-r93) [.org/10.1186/gb-2013-14-8-r93.](https://doi.org/10.1186/gb-2013-14-8-r93)
- 17. Kwan T, Liu J, DuBow M, Gros P, Pelletier J. 2005. The complete genomes and proteomes of 27 Staphylococcus aureus bacteriophages. Proc Natl Acad Sci U S A 102:5174-5179. [https://doi.org/10.1073/pnas.0501140102.](https://doi.org/10.1073/pnas.0501140102)
- 18. Culbertson EK, Bari SMN, Dandu VS, Kriznik JM, Scopel SE, Stanley SP, Lackey K, Hernandez AC, Hatoum-Aslan A. 2019. Draft genome sequences of Staphylococcus podophages JBug18, Pike, Pontiff, and Pabna. Microbiol Resour Announc 8:e00054-19. [https://doi.org/10.1128/](https://doi.org/10.1128/MRA.00054-19) [MRA.00054-19.](https://doi.org/10.1128/MRA.00054-19)
- 19. Vybiral D, Takáč M, Loessner M, Witte A, von Ahsen U, Bläsi U. 2003.

Complete nucleotide sequence and molecular characterization of two lytic Staphylococcus aureus phages: 44AHJD and P68. FEMS Microbiol Lett 219:275–283. [https://doi.org/10.1016/S0378-1097\(03\)00028-4.](https://doi.org/10.1016/S0378-1097(03)00028-4)

20. Holden MT, Feil EJ, Lindsay JA, Peacock SJ, Day NP, Enright MC, Foster TJ, Moore CE, Hurst L, Atkin R, Barron A, Bason N, Bentley SD, Chillingworth C, Chillingworth T, Churcher C, Clark L, Corton C, Cronin A, Doggett J, Dowd L, Feltwell T, Hance Z, Harris B, Hauser H, Holroyd S, Jagels K, James KD, Lennard N, Line A, Mayes R, Moule S, Mungall K, Ormond D, Quail MA, Rabbinowitsch E, Rutherford K, Sanders M, Sharp S, Simmonds M, Stevens K, Whitehead S, Barrell BG, Spratt BG, Parkhill J. 2004. Complete genomes of two clinical Staph-

ylococcus aureus strains: evidence for the rapid evolution of virulence and drug resistance. Proc Natl Acad Sci U S A 101:9786-9791. [https://doi.org/10.1073/pnas.0402521101.](https://doi.org/10.1073/pnas.0402521101)

- 21. Zaballos A, Salas M. 1989. Functional domains in the bacteriophage phi 29 terminal protein for interaction with the phi 29 DNA polymerase and with DNA. Nucleic Acids Res 17:10353–10366. [https://doi.org/10.1093/](https://doi.org/10.1093/nar/17.24.10353) [nar/17.24.10353.](https://doi.org/10.1093/nar/17.24.10353)
- 22. Watabe K, Leusch M, Ito J. 1984. Replication of bacteriophage phi 29 DNA in vitro: the roles of terminal protein and DNA polymerase. Proc Natl Acad Sci U S A 81:5374 –5378. [https://doi.org/10.1073/pnas.81.17](https://doi.org/10.1073/pnas.81.17.5374) [.5374.](https://doi.org/10.1073/pnas.81.17.5374)