AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of *Staphylococcus epidermidis* Myophages Quidividi, Terranova, and Twillingate

Miranda E. Freeman,[a] Sarah E. Kenny,[a] Amanda Lanier,[a] Katie Cater,[b] Mary C. Wilhite,[b] Paige Gamble,[b] Chandler J. O'Leary,[a] Asma Hatoum-Aslan,[b] Ryland F. Young III,[a] Mei Liu[a]

[a]Center for Phage Technology, Texas A&M University, College Station, Texas, USA
[b]Department of Biological Sciences, The University of Alabama, Tuscaloosa, Alabama, USA

ABSTRACT *Staphylococcus epidermidis* is an opportunistic pathogen that commonly colonizes human skin and mucous membranes. We report here the complete genome sequences of three *S. epidermidis* phages, Quidividi, Terranova, and Twillingate, which are members of the Twort-like group of large myophages infecting Gram-positive hosts.

Staphylococcus epidermidis is a Gram-positive opportunistic pathogen commonly associated with infections of implanted medical devices, and methicillin-resistant *S. epidermidis* (MRSE) continues to persist in both the health care and community environments (1, 2). Isolation and characterization of virulent phages active against *S. epidermidis* may provide an alternative control approach to this bacterium.

Quidividi and Terranova were isolated from a wastewater treatment plant in Tuscaloosa, Alabama, in 2016 with *S. epidermidis* strain RP62a (2) and strain LM1680, respectively, as the hosts. Twillingate was isolated from a wastewater treatment plant in Northport, Alabama, in 2016 using *S. epidermidis* strain RP62a (2) as the host. Host bacteria were cultured on tryptic soy broth or agar (Difco) at 37°C with aeration. Phages were cultured and propagated using the soft agar overlay method (3). The three phages were identified as myophages using negative-stain transmission electron microscopy performed at the University of Alabama Optical Analysis Facility as described previously (4). Phage genomic DNA was prepared using a modified Promega Wizard DNA cleanup kit protocol as described previously (4). Pooled indexed DNA libraries were prepared using the Illumina TruSeq Nano low-throughput (LT) kit, and sequencing was obtained from the Illumina MiSeq platform using the MiSeq V2 500-cycle reagent kit following the manufacturer's instructions, producing 539,431, 484,323, and 538,626 reads for the indexes containing Quidividi, Terranova, and Twillingate, respectively. FastQC 0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for quality control of the reads. The reads were trimmed with FastX-Toolkit 0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/download.html) before being assembled to single contigs at 139-, 107-, and 164-fold coverage for Quidividi, Terranova, and Twillingate, respectively, using SPAdes 3.5.0 (5). Contig completion was confirmed with PCR using primers (5′-AGAGGCTATCGCTCTTGAATTAG-3′ and 5′-TGTGTATATTGCTGTCGTGTAGAA-3′ for Quidividi; 5′-GTACAGGTTGGCATCCAGAAT-3′ and 5′-TCCGTGTGCTTGATTACCTTAC-3′ for Terranova; and 5′-TGAATAACTCTGTTAAACGGGCA-3′ and 5′-GGTCGTCACCCTTACGTTTAATT-3′ for Twillingate) facing off the ends of the assembled contigs and Sanger sequencing of the resulting product, followed by manual correction to match the sequencing reads. GLIMMER 3.0 (6) and MetaGeneAnnotator 1.0 (7) were used to predict the protein-coding genes, and tRNA genes were predicted using ARAGORN 2.36 (8). Rho-independent termination sites were identified via TransTerm (http://transterm.cbcb.umd.edu/). Sequence similarity searches were conducted using BLASTp

Citation Freeman ME, Kenny SE, Lanier A, Cater K, Wilhite MC, Gamble P, O'Leary CJ, Hatoum-Aslan A, Young RF, III, Liu M. 2019. Complete genome sequences of *Staphylococcus epidermidis* myophages Quidividi, Terranova, and Twillingate. Microbiol Resour Announc 8:e00598-19. https://doi.org/10.1128/MRA.00598-19.

Editor John J. Dennehy, Queens College

Address correspondence to Asma Hatoum-Aslan, asma.hatoum@ua.edu, or Mei Liu, meiliu@tamu.edu.

M.E.F., S.E.K., and A.L. contributed equally to this work.

Received 20 May 2019
Accepted 28 May 2019
Published 27 June 2019

2.2.28 (9) against the NCBI nonredundant (nr), UniProt Swiss-Prot (10), and TrEMBL databases. InterProScan 5.15-54.0 (11), LipoP (12), and TMHMM 2.0 (13) were used to predict protein function. All analyses were conducted at default settings via the CPT Galaxy (14) and Web Apollo (15) interfaces (https://cpt.tamu.edu/).

Myophages Quidividi, Terranova, and Twillingate possess similar genome sizes (141,446 bp, 141,288 bp, and 142,592 bp, respectively), coding densities (88% to 91%), and low GC contents (each has 28%). The phages share 83% to 88% DNA similarity and are >82% identical to phage phiIPLA-C1C (16), as determined by progressiveMauve 2.4.0 (17). These phages are members of the Twort-like group of large myophages, and they possess multiple self-splicing group I introns in their genomes (18). Introns with precise boundaries were identified in the terminase large subunit, ribonucleotide reductase subunit, double-strand break Mre1 repair protein, endolysin, and DNA polymerase genes. Introns were identified interrupting the tape measure protein-coding regions, but the precise boundaries could not be determined bioinformatically. An intein interrupting the DNA helicase was also identified.

Data availability. Quidividi has been deposited in GenBank under accession no. MH321490, SRA no. SRR8788536, and BioSample no. SAMN11260697. Terranova has been deposited in GenBank under accession no. MH542234, SRA no. SRR8788203, and BioSample no. SAMN11259659. Twillingate has been deposited in GenBank under accession no. MH321491, SRA no. SRR8788533, and BioSample no. SAMN11260686. These three phages are located under BioProject no. PRJNA222858.

ACKNOWLEDGMENTS

This work was supported by funding from the National Science Foundation (awards EF-0949351, DBI-1565146, and MCB-1749886). Additional support came from the Center for Phage Technology (CPT), from an Initial University Multidisciplinary Research Initiative supported by Texas A&M University and Texas AgriLife, and from the Department of Biochemistry and Biophysics at Texas A&M University.

We are grateful for the advice and support of the CPT staff and Kim Lackey at the University of Alabama Optical Analysis Facility. These phages were discovered through BSC 411 Phage Discovery, an undergraduate course at the University of Alabama, and this announcement was prepared in partial fulfillment of the requirements for BICH464 Phage Genomics, an undergraduate course at Texas A&M University.

REFERENCES

1. Otto M. 2009. Staphylococcus epidermidis—the "accidental" pathogen. Nat Rev Microbiol 7:555–567. https://doi.org/10.1038/nrmicro2182.
2. Gill SR, Fouts DE, Archer GL, Mongodin EF, Deboy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM. 2005. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant Staphylococcus aureus strain and a biofilm-producing methicillin-resistant Staphylococcus epidermidis strain. J Bacteriol 187:2426–2438. https://doi.org/10.1128/JB.187.7.2426-2438.2005.
3. Adams MK. 1959. Bacteriophages. Interscience Publishers, Inc., New York, NY.
4. Cater K, Dandu VS, Bari SM, Lackey K, Everett GF, Hatoum-Aslan A. 2017. A novel Staphylococcus podophage encodes a unique lysin with unusual modular design. mSphere 2:e00040-17. https://doi.org/10.1128/mSphere.00040-17.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. The UniProt Consortium. 2018. UniProt: the universal protein knowledgebase. Nucleic Acids Res 46:2699. https://doi.org/10.1093/nar/gky092.
11. Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong SY, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics 30:1236–1240. https://doi.org/10.1093/bioinformatics/btu031.
12. 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.
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. Cock PJ, Gruning 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.

15. 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.
16. Gutierrez D, Vandenheuvel D, Martinez B, Rodriguez A, Lavigne R, Garcia P. 2015. Two phages, phiIPLA-RODI and phiIPLA-C1C, lyse mono- and dual-species staphylococcal biofilms. Appl Environ Microbiol 81:3336–3348. https://doi.org/10.1128/AEM.03560-14.
17. 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.
18. Vandersteegen K, Kropinski AM, Nash JH, Noben JP, Hermans K, Lavigne R. 2013. Romulus and Remus, two phage isolates representing a distinct clade within the Twortlikevirus genus, display suitable properties for phage therapy applications. J Virol 87:3237–3247. https://doi.org/10.1128/JVI.02763-12.