




Whole-Genome Sequences of Phages p000v and p000y, Which Infect the Bacterial Pathogen Shiga-Toxigenic *Escherichia coli*

 Cristina Howard-Varona,^a Dean R. Vik,^a Natalie E. Solonenko,^a M. Consuelo Gazitua,^a Zack Hobbs,^b Ryan W. Honaker,^b Anika A. Kinkhabwala,^b Matthew B. Sullivan^{a,c}

^aDepartment of Microbiology, The Ohio State University, Columbus, Ohio, USA

^bEpiBiome, Inc., Union City, California, USA

^cDepartment of Civil, Environmental and Geodetic Engineering, The Ohio State University, Columbus, Ohio, USA

ABSTRACT We report here the genome sequences and morphological characterizations of phages p000v and p000y, which infect the bacterial pathogen Shiga-toxigenic *Escherichia coli* O157:H7 and which are potential candidates for phage therapy against such pathogens.

Antibiotic resistance in bacterial pathogens is growing at such an alarming rate throughout the world that the World Health Organization implores that researchers find alternatives to antibiotics to treat bacterial pathogens (1). One such solution is phage therapy, the use of bacteriophages against antibiotic-resistant bacteria (2). For Shiga-toxigenic *Escherichia coli* (STEC), which causes hemolytic uremic syndrome (3), antibiotic resistance is not only problematic but also can worsen the disease (4). Here, we present whole-genome sequences for two new phages that infect STEC O157:H7, p000v and p000y, as potential candidates for phage therapy against such pathogens.

Phages p000v and p000y were isolated from San Francisco, California, wastewater from overnight shaking cultures with exponentially growing laboratory *E. coli* cells in tryptone soya broth at 37°C, largely following previously described methods (5), and then isolated via the soft agar protocol (6, 7). The particles have *Myoviridae* morphology, as revealed by transmission electron microscopy (Fig. 1). Extraction and purification of genomic DNA was done from phage cultures using a phage DNA isolation kit (Norgen Biotek Corp., Thorold, ON, Canada) according to the manufacturer's protocol. The DNA library was prepared using the Nextera XT kit (Illumina, San Diego) according to the manufacturer's protocol (part number 15031942, revision D). The magnetic bead normalization step was replaced with a manual normalization step, based on library concentration and average size as measured by the Qubit 3.0 fluorometer and Qubit double-stranded DNA (dsDNA) high-sensitivity kit (Thermo Fisher Scientific, Waltham, MA) and the Fragment Analyzer (AATI, Ankeny, IA), respectively. Whole-phage DNA was sequenced with a MiSeq next-generation sequencing platform, using the MiSeq reagent v3 (600-cycle) kit (Illumina, San Diego, CA). A total of 100,000 paired reads of 300 bp without any quality trimming were assembled *de novo* using the SPAdes 3.9.1 genome assembler software using default parameters (8), with which one contig was obtained for each phage genome. The coverages for the genomes of phages p000v and p000y were 178.7× and 176.6×, respectively. Open reading frame prediction and annotation was done with the Prokka 1.12-beta software (9) and the *-kingdom viruses* option.

Phage p000v has a genome size of 167.9 kbp, with 37.6% GC content, and phage p000y has a genome size of 169.9 kbp and 37.7% GC content. Both phages share 96.7% identity, obtained via a whole-genome blastn 2.2.31 (10) analysis with an *e* value

Received 18 October 2018 **Accepted** 22 October 2018 **Published** 21 November 2018

Citation Howard-Varona C, Vik DR, Solonenko NE, Gazitua MC, Hobbs Z, Honaker RW, Kinkhabwala AA, Sullivan MB. 2018. Whole-genome sequences of phages p000v and p000y, which infect the bacterial pathogen Shiga-toxigenic *Escherichia coli*. *Microbiol Resour Announc* 7:e01400-18. <https://doi.org/10.1128/MRA.01400-18>.

Editor Catherine Putonti, Loyola University Chicago

Copyright © 2018 Howard-Varona et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Matthew B. Sullivan, sullivan.948@osu.edu.

C.H.-V. and D.R.V. contributed equally to this work.

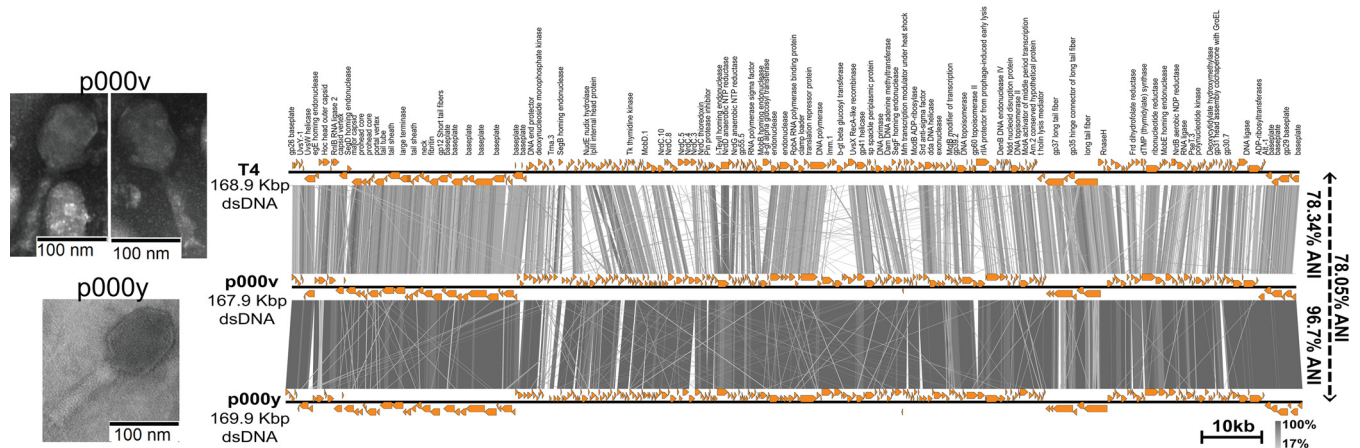


FIG 1 Morphological and genetic characterization of phages p000v and p000y. Whole-genome comparison of p000v, p000y, and *E. coli* phage T4 reveals that p000v and p000y are T4-like due to their high average nucleotide identity (ANI) shared with T4. For T4, GenBank sequence [AF158101](#) was used. Shown via EasyFig 2.2.2 (10) are the percent protein similarities, obtained via a tblastx search (16) with an e value of 0.001, and their average nucleotide identities. Next to each genome is a transmission electron microscopy (TEM)-derived micrograph, which reveals that both phages have a *Myoviridae* morphology. TEM was performed at The Ohio State University (OSU) Campus Microscopy and Imaging Facility (CMIF) on an FEI Tecnai G2 Spirit TEM at an acceleration voltage of 80 kV, following a modified protocol (17). Samples were derived from 0.2- μ m-filtered phage lysates that were concentrated with polyethylene glycol (p000v) and also purified with a CsCl (p000y) density gradient and dialyzed in phage buffer. For phage p000v, a 10- μ l sample was deposited onto Formvar-coated 200-mesh copper TEM grids (Ted Pella, Inc., Redding, CA) and incubated for 30 min at room temperature. Grids were washed twice with distilled water and negatively stained with 10 μ l of 2% uranyl acetate for 15 s. For p000y, a 200- μ l sample was deposited onto the grids with an air-driven ultracentrifuge (Airfuge CLS, Beckman Coulter, Brea, CA), followed by negative staining as described.

threshold of 0.0001. Additionally, both phages share >78% average nucleotide identity (ANI) with coliphage T4 (GenBank reference sequence [AF158101](#)) (11) (Fig. 1), obtained via the ANI calculator developed by Goris et al. (12). The shared genes included 38 T4-like phage core genes (defined by Sullivan et al. [13], with which a database was created) at >81% average identity, obtained via a blastn 2.2.31 (10) analysis. Both phages p000y and p000v appear to be T4-like myoviruses, based on conventional affiliation criteria (13, 14).

Data availability. Raw reads and assembled genome sequences of phages p000v and p000y are available in GenBank under the accession numbers [MK047717](#) and [MK047718](#), respectively, and on Cyverse (15) under DOI 10.7946/P2HP89 (<https://www.doi.org/10.7946/P2HP89>).

ACKNOWLEDGMENTS

We thank Michelle Davison and Rebecca Lu at EpiBiome for DNA extraction and sequencing, respectively.

Partial support comes from the Infectious Diseases Institute Seed Grant program through The Ohio State University (OSU) to M.B.S.; NIH/NIAID award 1-T32-AI-112542 to C.H.-V. through the Infectious Disease Institute at OSU; and the Bill & Melinda Gates Foundation Grand Challenges Award OPP1139917 to EpiBiome.

REFERENCES

- Collignon PC, Conly JM, Andremont A, McEwen SA, Aidara-Kane A, World Health Organization Advisory Group, Bogotà Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR), Agerso Y, Andremont A, Collignon P, Conly J, Dang Ninh T, Donado GP, Fedorka CP, Fernandez H, Galas M, Irwin R, Karp B, Matar G, McDermott P, McEwen S, Mitema E, Reid SR, Scott HM, Singh R, DeWaal CS, Stelling J, Toleman M, Watanabe H, Woo G-J. 2016. World Health Organization ranking of antimicrobials according to their importance in human medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. *Clin Infect Dis* 63: 1087–1093. <https://doi.org/10.1093/cid/ciw475>.
- Harada LK, Silva EC, Campos WF, Del Fiol FS, Vila M, Dąbrowska K, Krylov VN, Balcão VM. 2018. Biotechnological applications of bacteriophages: state of the art. *Microbiol Res* 212–213:38–58. <https://doi.org/10.1016/j.micres.2018.04.007>.
- Smith JL, Fratamico PM. 2012. Effect of stress on non-O157 Shiga toxin-producing *Escherichia coli*. *J Food Prot* 75:2241–2250. <https://doi.org/10.4315/0362-028X.JFP-12-255>.
- Los JM, Los M, Wegrzyn G. 2011. Bacteriophages carrying Shiga toxin genes: genomic variations, detection and potential treatment of pathogenic bacteria. *Future Microbiol* 6:909–924. <https://doi.org/10.2217/FMB.11.70>.
- Callaway TR, Edrington TS, Brabban AD, Keen JE, Anderson RC, Rossman ML, Engler MJ, Genovese KJ, Gwartney BL, Reagan JO, Poole TL, Harvey RB, Kutter EM, Nisbet DJ. 2006. Fecal prevalence of *Escherichia coli* O157, *Salmonella*, *Listeria*, and bacteriophage infecting *E. coli* O157:H7 in feed-

- lot cattle in the Southern Plains region of the United States. *Foodborne Pathog Dis* 3:234–244. <https://doi.org/10.1089/fpd.2006.3.234>.
6. Sambrook J, Russell DW. 2000. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
 7. Holmfeldt K, Howard-Varona C, Solonenko N, Sullivan MB. 2014. Contrasting genomic patterns and infection strategies of two co-existing *Bacteroidetes* podovirus genera. *Environ Microbiol* 16:2501–2513. <https://doi.org/10.1111/1462-2920.12391>.
 8. 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>.
 9. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
 10. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403–410. [https://doi.org/10.1016/S0022-2836\(05\)80360-2](https://doi.org/10.1016/S0022-2836(05)80360-2).
 11. Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, Ruger W. 2003. Bacteriophage T4 genome. *Microbiol Mol Biol Rev* 67:86–156. <https://doi.org/10.1128/MMBR.67.1.86-156.2003>.
 12. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijs.0.64483-0>.
 13. Sullivan MB, Huang KH, Ignacio-Espinoza JC, Berlin AM, Kelly L, Weigele PR, DeFrancesco AS, Kern SE, Thompson LR, Young S, Yandava C, Fu R, Krastins B, Chase M, Sarracino D, Osburne MS, Henn MR, Chisholm SW. 2010. Genomic analysis of oceanic cyanobacterial myoviruses compared with T4-like myoviruses from diverse hosts and environments. *Environ Microbiol* 12:3035–3056. <https://doi.org/10.1111/j.1462-2920.2010.02280.x>.
 14. Lavigne R, Darius P, Summer EJ, Seto D, Mahadevan P, Nilsson AS, Ackermann HW, Kropinski AM. 2009. Classification of *Myoviridae* bacteriophages using protein sequence similarity. *BMC Microbiol* 9:224. <https://doi.org/10.1186/1471-2180-9-224>.
 15. Merchant N, Lyons E, Goff S, Vaughn M, Ware D, Micklos D, Antin P. 2016. The iPlant Collaborative: cyberinfrastructure for enabling data to discovery for the life sciences. *PLoS Biol* 14:e1002342. <https://doi.org/10.1371/journal.pbio.1002342>.
 16. Sullivan MJ, Petty NK, Beatson SA. 2011. Easyfig: a genome comparison visualizer. *Bioinformatics* 27:1009–1010. <https://doi.org/10.1093/bioinformatics/btr039>.
 17. Shan J, Patel KV, Hickenbotham PT, Nale JY, Hargreaves KR, Clokie MRJ. 2012. Prophage carriage and diversity within clinically relevant strains of *Clostridium difficile*. *Appl Environ Microbiol* 78:6027–6034. <https://doi.org/10.1128/AEM.01311-12>.