



Complete Genome Sequence of *Escherichia* Phage vB_EcoM-Pr121LW, Isolated from Soil in an Organic Farm

Yen-Te Liao,^a Fang Liu,^{a,b} ^(D) Vivian C. H. Wu^a

^aProduce Safety and Microbiology Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, Albany, California, USA ^bCollege of Food Science and Engineering, Ocean University of China, Qingdao, China

ABSTRACT Here, we report a new member of rV5-like phages, *Escherichia* phage vB_EcoM-Pr121LW, isolated from soil samples and lytic against different serogroups of Shiga toxin-producing *Escherichia coli* (STEC) strains. With molecular properties that contain no antibiotic resistance, virulence, or lysogenic genes, this phage is a potential biocontrol agent against STEC.

Shiga toxin-producing *Escherichia coli* (STEC) strains have been recently associated with the increasing number of produce outbreaks (1, 2). Bacteriophages have shown their potential as alternative biocontrol agents to replace antibiotics or preservatives against these pathogens (3). In the current study, the complete genome sequence of *Escherichia* phage vB_EcoM-Pr121LW, which is lytic against various STEC strains, is described.

Escherichia phage vB_EcoM-Pr121LW (or Pr121LW) was isolated from a soil sample collected in an organic farm by enriching with STEC O121 strains in tryptic soy broth with 10% calcium chloride solution and incubating at 37°C overnight, followed by the purification process described previously, with subtle modification (4). The phage DNA was extracted using a phage DNA isolation kit (Norgen Biotek, Ontario, Canada), and the libraries were prepared as previously described (5). Briefly, a TruSeq Nano DNA library prep kit (Illumina) was used for the library preparation, with quantification performed using an Agilent 2100 Bioanalyzer. Approximately 2.5 million 2×250 -bp paired-end sequence reads were generated using a MiSeq sequencer, according to the manufacturer's instruction. The raw sequence reads were subjected to FastQC for quality check, followed by sequence trimming using Trimmomatic, with default settings. The resultant quality reads were assembled to a single contig using Unicycler version 0.4.1 (SPAdes) on the Galaxy server (https://usegalaxy.org/). The open reading frames (ORFs) of the final contig were predicted using Geneious (version 11.0.4) with a reference genome and confirmed with the BLASTp results. The phage packaging mechanisms and genome termini were predicted using PhageTerm (6). tRNA was detected using the tRNAscan-SE search server (7). Antibiotic resistance genes were examined on the ResFinder (version 3.0) database (8).

Pr121LW contained a 134,575-bp double-stranded DNA (224-fold coverage), with an average G+C content of 43.6%, and was classified as a novel rV5-like virus, with 96% sequence identity over 96% coverage of the phage rV5 (GenBank accession number DQ832317), under subfamily *Vequintavirinae* within *Myoviridae*, according to NCBI BLASTn analysis. Genome annotation predicted 220 open reading frames (ORFs), of which 50 encoded functional proteins, including those for DNA replication and metabolism, cell lysis, and structural integrity. No antibiotic resistance genes, lysogenic genes, or virulence genes, such as *stx* and *eaeA*, were found. Pr121LW also shared 98% sequence identity with approximately 98% of an rV5-like phage genome, that of

Received 21 September 2018 Accepted 24 October 2018 Published 21 November 2018

Citation Liao Y-T, Liu F, Wu VCH. 2018. Complete genome sequence of *Escherichia* phage vB_EcoM-Pr121LW, isolated from soil in an organic farm. Microbiol Resour Announc 7:e01236-18. https://doi.org/10.1128/MRA .01236-18.

Editor John J. Dennehy, Queens College This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Vivian C. H. Wu, vivian.wu@ars.usda.gov. *Escherichia* phage Murica (GenBank accession number KT001917). However, a 423-bp noncoding region interrupted a large terminase in Pr121LW, unlike the complete terminase sequence in phage Murica. Additionally, Pr121LW had 5 tRNA genes, whereas Murica had 7 (9). PhageTerm predicted that the packaging mechanism of Pr121LW was headful with a *pac* site, without terminal cohesive ends (10).

Most rV5-like phages, including Murica, were categorized in a cluster of lytic phages, Lytic22, among 337 fully sequenced tail phages based on comparative genomic analyses (11). Furthermore, three tail fiber proteins of Pr121LW (ORF_117, ORF_122, and ORF_131) show 99% identity to the amino acid sequences of another rV5-like phage, vB_EcoM_FFH2 (GenBank accession number NC_024134), which had a wide host range against STEC strains (12). The genomic analyses were consistent with the wide host range of Pr121LW.

The findings of this study demonstrate that Pr121LW may be a new member of rV5-like phages isolated from a nonfecal environment. Additionally, the genomic features provide valuable insights into the use of a potential biocontrol agent against STEC strains.

Data availability. The complete genome sequence of *Escherichia* phage vB_EcoM-Pr121LW has been deposited in GenBank under the accession number MH752840. The sequencing reads have been deposited under the accession number SRP159220. The version of the phage genome described in this paper is the first version.

ACKNOWLEDGMENTS

We thank Leslie Harden, Valerie Lavenburg, Marion Lennon, and David Bridges at the USDA-ARS-WRRC Produce Safety and Microbiology Research Unit for the sample collection.

This research was funded by the U.S. Department of Agriculture, Agricultural Research Service (CRIS project 2030-42000-050-00D).

REFERENCES

- Beutin L, Martin A. 2012. Outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. J Food Prot 75:408–418. https://doi.org/10.4315/0362-028X.JFP-11-452.
- Herman KM, Hall AJ, Gould LH. 2015. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. Epidemiol Infect 143:3011–3021. https://doi.org/10.1017/S0950268815000047.
- Mahony J, McAuliffe O, Ross RP, van Sinderen D. 2011. Bacteriophages as biocontrol agents of food pathogens. Curr Opin Biotechnol 22:157–163. https://doi.org/10.1016/j.copbio.2010.10.008.
- 4. Liao Y-T, Quintela IA, Nguyen K, Salvador A, Cooley MB, Wu VCH. 2018. Investigation of prevalence of free Shiga toxin- producing *Escherichia coli* (STEC)-specific bacteriophages and its correlation with STEC bacterial hosts in a produce-growing area in Salinas, California. PLoS One 13:e0190534. https://doi.org/10.1371/journal.pone.0190534.
- Liao Y-T, Liu F, Sun X, Li RW, Wu VCH. 2018. Complete genome sequence of *Escherichia coli* phage vB_EcoS Sa179lw, isolated from surface water in a produce-growing area in northern California. Genome Announc 6:e00337-18. https://doi.org/10.1128/genomeA.00337-18.
- Garneau JR, Depardieu F, Fortier LC, 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.

- Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44: W54–W57. https://doi.org/10.1093/nar/gkw413.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi .org/10.1093/jac/dks261.
- Wilder JN, Lancaster JC, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of enterotoxigenic *Escherichia coli* myophage Murica. Genome Announc 3:e01135-15. https://doi.org/10 .1128/genomeA.01135-15.
- Coren JS, Pierce JC, Sternberg N, Sternberg N. 1995. Headful packaging revisited: the packaging of more than one DNA molecule into a bacteriophage P1 head. J Mol Biol 249:176–184. https://doi.org/10.1006/jmbi .1995.0287.
- Grose JH, Casjens SR. 2014. Understanding the enormous diversity of bacteriophages: the tailed phages that infect the bacterial family *Enterobacteriaceae*. Virology 468–470:421–443. https://doi.org/10.1016/j.virol .2014.08.024.
- Hong Y, Pan Y, Harman NJ, Ebner PD. 2014. Complete genome sequences of two *Escherichia coli* O157:H7 phages effective in limiting contamination of food products. Genome Announc 2:e00519-14. https:// doi.org/10.1128/genomeA.00519-14.