



Complete Genome Sequence of a Shiga Toxin-Converting Bacteriophage, *Escherichia* Phage Lys12581Vzw, Induced from an Outbreak Shiga Toxin-Producing *Escherichia coli* Strain

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ABSTRACT Although numerous Shiga toxin (Stx)-producing *Escherichia coli* (STEC) strains have been sequenced, genomic information on Stx-converting phages, highly related to the primary virulence factors of STEC, is scarce. Here, we report the complete genome sequence of a Stx-converting phage induced from an outbreak STEC O145 strain.

Shiga toxin (Stx)-producing *Escherichia coli* (STEC), as one of the major foodborne pathogens, has been widely associated with multiple foodborne outbreaks in the United States (1, 2). Stx-converting phages, carrying the major virulence genes of STEC, are usually induced from STEC strains and have been related to the emergence of new STEC strains through the transfer of *stx* genes to other bacteria (3, 4). However, there is a limited number of complete whole-genome sequences of Stx-converting phages. In this study, the complete genome sequence of a Stx2-converting phage isolated from an outbreak STEC O145 strain is described.

The Stx-converting phage Lys12581Vzw was induced from *E. coli* O145:H28 (RM12581), associated with the U.S. romaine lettuce outbreak in 2010 (5), by using mitomycin C (0.5 μ g/ml) in LB broth at 37°C overnight. Phage purification was performed using a double-layer plaque assay against *E. coli* strain WG5 (ATCC 700078) from a single plaque picking, followed by CsCl gradient concentration to get rid of bacterial DNA and debris. The phage DNA was extracted using a phage DNA extraction kit (Norgen Biotek, Ontario, Canada). The DNA library (2 \times 250 bp) was constructed using a TruSeq Nano DNA library prep kit (Illumina, San Diego, CA) and was subsequently sequenced on an Illumina MiSeq sequencer, resulting in 6,779,298 paired-end reads. The raw sequence reads were quality filtered and trimmed using FastQC and Trimmomatic (6), with the average quality set at Q30. *De novo* assembly of the remaining quality reads was performed using SPAdes version 3.13.0 on the KBase server (7, 8) with the minimum contig length set to >10,000 bp. The resulting contig was annotated using both Prokka (9) and RAST server (10) pipelines with default settings. The annotations were subsequently compared and curated with UniProt (11) and BLASTp (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins>), with the identity and query coverage greater than 95%, using Geneious (version 11.0.4). The packaging mechanisms and genome termini were determined using PhageTerm (12). tRNAs were predicted using the tRNAscan-SE search server (13). ResFinder (version 3.0) and VirulenceFinder were used to screen for antibiotic resistance genes and virulence genes (14, 15).

Phage Lys12581Vzw, belonging to the family *Podoviridae*, has double-stranded DNA with a genome size of 62,668 bp (3,257 \times coverage) and an average G+C content of 50.1%. The phage has a Mu-like packaging mechanism; the phage genome lacks

Citation Zhang Y, Liao Y-T, Salvador A, Sun X, Wu VCH. 2019. Complete genome sequence of a Shiga toxin-converting bacteriophage, *Escherichia* phage Lys12581Vzw, induced from an outbreak Shiga toxin-producing *Escherichia coli* strain. *Microbiol Resour Announc* 8:e00793-19. <https://doi.org/10.1128/MRA.00793-19>.

Editor John J. Dennehy, Queens College

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Received 30 June 2019

Accepted 15 August 2019

Published 5 September 2019

obvious termini and carries pieces of the host sequences. There are 81 open reading frames (ORFs) predicted, of which 45 are annotated with functional proteins, such as DNA replication and modification (restriction endonuclease, DNA primase, and methylase), membrane-related proteins (lysis proteins, membrane proteins, and cell division inhibitors), antirepressors, virion structures, integrases, Shiga toxin, and tRNAs. Most functional ORFs contribute to the virulence and propagation of Stx-converting phages. None of the ORFs contain antibiotic resistance genes. The whole-genome sequence of Lys12581Vzw shares 95% average nucleotide identity with two published Stx2-converting phages—*Enterobacteria* phage VT2-Sakai (GenBank accession number [NC_000902](#)) and bacteriophage 933W (GenBank accession number [AF125520](#))—induced from different *E. coli* O157:H7 strains. This study provides valuable insights into the diversity of Stx-converting phages which are associated with the evolution of STEC strains.

Data availability. The complete genome sequence of *Escherichia* phage Lys12581Vzw has been deposited in GenBank under the accession number [MN067333](#). The sequencing reads have been deposited under the BioSample accession number [SAMN12086808](#). The version of the phage genome described in this paper is the first version.

ACKNOWLEDGMENT

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

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