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GENOME SEQUENCES

# Complete Genome Sequence of *Enterobacter* Phage vB_EcRAM-01, a New *Pseudotevenvirus* against the *Enterobacter cloacae* Complex

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**ABSTRACT** Here, we present the complete genome sequence of *Enterobacter* phage vB_EcRAM-01, isolated from waters of the Río Abajo river, in Panama City, Panama. This phage has deployed lytic activity against the *Enterobacter cloacae* complex, a pathogen of clinical importance in intensive care units. It belongs to the *Myoviridae* family and has a double-stranded DNA genome that is 178,477 bp long and contains 293 open reading frames (ORFs).

The *Enterobacter cloacae* complex has been classified by the World Health Organization (WHO) as a priority for antimicrobial research and development (1). In intensive care units, phage therapy has become a viable option for treating multidrug-resistant bacteria, among which *E. cloacae* represents a serious threat (2, 3).

The *Enterobacter* phage vB_EcRAM-01 was isolated from waters of the Rio Abajo river in Panama City, Panama (coordinates, 9°00′55.2″N 79°29′25.6″W), using *E. cloacae* strain ATCC 23355 as a propagation host, which was grown previously in tryptic soy broth at 37°C for 24 h (4, 5). Phage isolation, propagation, and purification were performed using the soft-agar overlay method (6). DNA extraction was performed using the QIAamp MinElute virus spin kit (Qiagen, Valencia, CA, USA), according to the manufacturer's instructions. The DNA libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA) through tagmentation, PCR amplification, PCR cleanup, and library normalization. Genome sequencing was performed with the Illumina MiSeq platform (2 × 250-bp paired-end protocol, 300 cycles). Raw reads (629,372 reads in total) were checked for quality using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc) and trimmed using Trimmomatic v0.39 (7). The genome was assembled through SPAdes v3.15.1 (8), resulting in a single contig with 8.96-fold coverage. We mapped the reads against the resulting contig to confirm sequence ends using Bowtie (9). Alignments with closely related phages showed the presence of the same annotated features which indicates this single contig represents the complete genome. The genome was initially annotated with the fast annotation algorithm using RASTk v2.0 subsystem technology (10–12). The putative open reading frames (ORFs) were verified using GeneMarkS v4.28 (13, 14) and Glimmer v3.02 (15, 16).

The ORF functions were annotated using the protein basic local alignment search tool (blastp) of the NCBI server (https://blast.ncbi.nlm.nih.gov/Blast.cgi) based on a search of the nonredundant protein sequence database (17), HHpred using the structural/domain

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database (https://toolkit.tuebingen.mpg.de/tools/hhpred) (18, 19), and HMMER v2.41.1 through the HMM database (20, 21).

vB_EcRAM-01 has a 178,477-bp genome containing 293 genes, of which 86 have a predicted function associated with morphology, inactivation, cell adsorption, DNA injection, and host lysis. No resistance (ResFinder 4.1, https://cge.cbs.dtu.dk/services/ResFinder/) (22–24), virulence (VirulenceFinder 2.0, https://cge.cbs.dtu.dk/services/VirulenceFinder/) (25, 26), or allergenicity-related (AllergenOnline, http://www.allergenonline.org/databasefasta.shtml) (27) genes were identified. The raw reads were assembled, automatically annotated, and manually curated. This genome codes for 2 tRNAs, namely, a tRNA-Met (cat) and a tRNA-Gly (tcc). *Enterobacter* phage vB_EcRAM-01 is most closely related to *Cronobacter* phage vB_CsaM_IeE (GenBank accession number NC_048646.1) with 90.11% nucleotide sequence identity determined by pyani v0.2.11 (28). Therefore, *Enterobacter* phage vB_EcRAM-01 is a new member of the *Pseudotevenvirus* genus within the *Tevenvirinae* subfamily and *Myoviridae* family, according to the genus demarcation criteria (>70% nucleotide identity over the full genome length) recommended by the ICTV Bacterial and Archaeal Viruses Subcommittee (29).

vB_EcRAM-01 exhibited specific litic activity against 19 clinical *E. cloacae* isolates.

The complete annotation of the vB_EcRAM-01 genome can be found in the GenBank database (30).

**Data availability.** The complete sequence of *Enterobacter* phage vB_EcRAM-01 has been deposited in GenBank under accession number OL551674 and BioSample accession SAMN23426491. Raw data can be accessed through SRA number SRR18344380.

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