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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Cronobacter sakazakii* Bacteriophage vB_CsaM_GAP161

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***Cronobacter sakazakii* is an opportunistic pathogen that causes infant meningitis and is often associated with milk-based infant formula. We have fully sequenced the genome of a newly isolated lytic *C. sakazakii* myovirus, vB_CsaM_GAP161, briefly named GAP161. It consists of 178,193 bp and has a G+C content of 44.5%. A total of 277 genes, including 275 open reading frames and two tRNA-encoding genes, were identified. This phage is closely related to coliphages RB16 and RB43 and *Klebsiella pneumoniae* phage KP15.**

Contaminated milk-based powdered infant formulae have been the source of *Cronobacter* infections that cause sepsis, brain abscess, and meningitis in neonates and infants (2, 5), with mortality up to 80% (7). Because of their high specificity and effectiveness, bacteriophages have been used as alternative agents to control pathogens (4, 6) and may be particularly relevant for the control of *Cronobacter* because of its intrinsic antibiotic resistance (7). However, complete knowledge of a potential therapeutic phage is required to ensure its safety before clinical application. Currently, there are only seven reported fully sequenced *Cronobacter* phages, including three members of the *Myoviridae* (CR3, ESSI-2, and ES2) (11, 13, 15), three members of the *Siphoviridae* (phiES15, ESP2949-1, and ENT39118) (9, 10, 12), and ENT47670 (unclassified; GenBank accession number HQ201308).

Lytic bacteriophages against *Cronobacter sakazakii* were isolated from sewage samples (Guelph, ON, Canada) using the method described by Van Twest and Kropinski (17). Phage vB_CsaM_GAP161, briefly named GAP161, lysed 12 of 14 *C. sakazakii* strains tested.

Based on host range and its strong lytic activity, phage GAP161 was chosen for further study. Electron microscopy of negatively stained (2% uranyl acetate) viral preparations was carried out at the University of Guelph, with sizes verified at Laval University. GAP161 belongs to the *Myoviridae* family (1), with an elongated cylindrical head that is 110 by 74 nm and a tail that is 113 by 17 nm, and has the characteristic morphology of a T4-like phage.

The DNA was extracted and purified by the Midi Lambda DNA purification kit (Qiagen, Mississauga, ON, Canada), and the genomic sequence was determined using 454 technology (McGill University and the Genome Quebec Innovation Centre, Montreal, QC, Canada). The genome was annotated using MyRAST, with gene calls verified using Kodon (Applied Maths). Transfer RNAs were predicted using tRNAscan-SE (http://lowelab.ucsc.edu/tRNAscan-SE/). For each protein, the number of amino acids, molecular weight, and isoelectric point were calculated using Batch MW and pI Finder (http://greengene.uml.edu/programs/FindMW.html). Homologs were identified using BatchBLAST (http://greengene.uml.edu/programs/NCBI_Blast.html). Transmembrane helices in proteins and transmembrane topology and signal peptides were predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and Phobius (http://phobius.sbc.su.se), respectively.

Phage GAP161 has a double-stranded DNA genome of 178,193 bp with a G+C content of 44.5%. This genome encodes 277 genes, including 275 open reading frames (ORFs) and two tRNA genes. Bioinformatic analysis using CoreGenes (18) showed that the genome sequence of GAP161 shares 94.07% (254/270), 87.21% (225/258), and 85.27% (249/292) homology with coliphages RB16 and RB43 (14) and phage KP15 of *Klebsiella pneumoniae* (3), respectively, indicating that GAP161 is a member of the myoviral subfamily *Teequatrovirinae* in a genus of T4-like viruses (8).

The complete proteome of phage GAP161 was screened against a database of 83 bacterial toxin proteins (including those from *Bacillus* spp., *Bordetella*, *Clostridium* spp., *Enterobacteriaceae*, *Listeria*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Vibrio*) using the BLASTP feature of BioEdit (16). No hits (E value < 0.003) were recorded, suggesting that phage GAP161 may be applied as a biocontrol agent against *Cronobacter sakazakii*.

Nucleotide sequence accession number. The complete genome sequence of phage vB_CsaM_GAP161 is available in GenBank under accession number JN882287.

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