

Genome Sequence of *Cronobacter sakazakii* Myovirus vB_CsaM_GAP31

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***Cronobacter sakazakii* is a pathogen that predominantly infects immunocompromised individuals, especially infants, where it causes meningitis. The genome of lytic *C. sakazakii* myovirus vB_CsaM_GAP31 has been fully sequenced. It consists of 147,940 bp and has a G+C content of 46.3%. A total of 295 genes, including 269 open reading frames and 26 tRNA genes, were identified. This phage is related to *Salmonella* phage PVP-SE1 and coliphages vB_EcoM-FV3 and rV5.**

Cronobacter sakazakii contaminations in milk-based powdered infant formulae have been the source of infections that cause sepsis, brain abscess, and meningitis in neonates and infants (2, 4), with mortality up to 80% (6). Bacteriophages (phages), due to their high specificity and effectiveness, have been used as alternative agents to control pathogens (3, 5) and could be particularly interesting for the control of *Cronobacter* because of its intrinsic antibiotic resistance (6). Comprehensive knowledge of a potential therapeutic phage, however, is required to ensure its safety before clinical application. To date, only seven fully sequenced *Cronobacter* phages have been reported, including three myoviruses (ESSI-2, ES2, and CR3) (10, 12, 14), three siphoviruses (ESP2949-1, phiES15, and ENT39118) (8, 9, 11), and ENT47670 (unclassified; GenBank accession number HQ201308).

Lytic *C. sakazakii* phages were isolated from sewage samples (Guelph, ON, Canada) by applying the method described by Van Twest and Kropinski (17). Phage vB_CsaM_GAP31 (GAP31) lysed 11 of 14 *C. sakazakii* strains tested. Phage GAP31 was chosen for further study because of its host range and strong lytic activity. Electron microscopy of negatively stained (2% uranyl acetate) viral preparations was carried out at the University of Guelph, and particle sizes were verified at Laval University. GAP31 is a myovirus (1) with an icosahedral head 83 nm in diameter and a tail of 107 by 19 nm.

Phage DNA was extracted and purified using the Midi Lambda DNA purification kit (Qiagen, Mississauga, ON, Canada). The genomic sequence was determined using 454 technology (McGill University and Génome Québec Innovation Centre, Montreal, QC, Canada). MyRAST was used to annotate the genome, and gene calls were verified in Kodon (Applied Maths, Austin, TX). The number of amino acids, molecular weight, and isoelectric point of each protein were determined 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). Protein motifs were predicted using Pfam (<http://pfam.sanger.ac.uk/search#tabview=tab1>), TMHMM (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>), and Phobius (<http://phobius.sbc.su.se>).

The double-stranded DNA genome of phage GAP31 has 147,940 bp with a G+C content of 46.3%. This genome contains

295 genes, including 269 open reading frames (ORFs) and 26 tRNA genes. Comparative proteomic analysis using CoreGenes (18) shows that the genome sequence of GAP31 encodes 202/244 (82.79%), 96/218 (44.04%), and 98/233 (42.06%) proteins that are homologs of those encoded by *Salmonella* phage PVP-SE1 (13) and coliphages vB_EcoM-FV3 (16) and rV5 (DQ832317), respectively. In addition, this virus is peripherally related to *Cronobacter* phage CR3, with 35.47% homology (94/265 proteins) (14). Phage GAP31 belongs to the proposed genus “V5likevirus” within the *Myoviridae* family (7).

The proteome of phage GAP31 was screened against a database of 83 bacterial toxin proteins (including those from *Bacillus*, *Bordetella*, *Clostridium*, *Enterobacteriaceae*, *Listeria*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, and *Vibrio* spp.) using the BLASTP feature of BioEdit (15). No hits (E value <0.003) were recorded, suggesting that phage GAP31 could be used as a biocontrol agent against *Cronobacter sakazakii*.

Nucleotide sequence accession number. The complete genome sequence of phage vB_CsaM_GAP31 is available in GenBank under accession number JN882284.

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REFERENCES

- Ackermann H-W. 2005. Bacteriophage classification, p 67–89. In Kutter E, Sulakvelidze A (ed), *Bacteriophages: biology and applications*. CRC Press, Boca Raton, FL.
- Centers for Disease Control and Prevention. 2002. *Enterobacter sakazakii* infections associated with the use of powdered infant formula—Tennessee, 2001. *MMWR Morb. Mortal. Wkly. Rep.* 51:297–300.
- Goodridge LD, Bisha B. 2011. Phage-based biocontrol strategies to reduce foodborne pathogens in foods. *Bacteriophage* 1:130–137. doi:10.4161/bact.1.3.17629.

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4. Gurtler JB, Kornacki JL, Beuchat LR. 2005. *Enterobacter sakazakii*: a coliform of increased concern to infant health. *Int. J. Food Microbiol.* **104**:1–34.
5. Kropinski AM. 2006. Phage therapy—everything old is new again. *Can. J. Infect. Dis. Med. Microbiol.* **17**:297–306.
6. Lai KK. 2001. *Enterobacter sakazakii* infections among neonates, infants, children, and adults—case reports and a review of the literature. *Medicine* **80**:113–122.
7. Lavigne R, et al. 2009. Classification of *Myoviridae* bacteriophages using protein sequence similarity. *BMC Microbiol.* **9**:224. doi:10.1186/1471-2180-9-224.
8. Lee Y-D, Kim J-Y, Park J-H, Chang H. 2012. Genomic analysis of bacteriophage ESP2949-1, which is virulent for *Cronobacter sakazakii*. *Arch. Virol.* **157**:199–202.
9. Lee JH, Choi Y, Shin H, Lee J, Ryu S. 2012. Complete genome sequence of *Cronobacter sakazakii* temperate bacteriophage phiES15. *J. Virol.* **86**:7713–7714.
10. Lee Y-D, Chang HI, Park J-H. 2011. Complete genomic sequence of virulent *Cronobacter sakazakii* phage ESS1-2 isolated from swine feces. *Arch. Virol.* **156**:721–724.
11. Lee Y-D, Park J-H. 2012. Complete genome of temperate phage ENT39118 from *Cronobacter sakazakii*. *J. Virol.* **86**:5400–5401.
12. Lee Y-D, Park J-H, Chang HI. 2011. Genomic sequence analysis of virulent *Cronobacter sakazakii* bacteriophage ES2. *Arch. Virol.* **156**:2105–2108.
13. Santos SB, et al. 2011. Genomic and proteomic characterization of the broad-host-range *Salmonella* phage PVP-SE1: creation of a new phage genus. *J. Virol.* **85**:11265–11273.
14. Shin H, Lee JH, Kim Y, Ryu S. 2012. Complete genome sequence of *Cronobacter sakazakii* bacteriophage CR3. *J. Virol.* **86**:6367–6368.
15. Tippmann HF. 2004. Analysis for free: comparing programs for sequence analysis. *Brief. Bioinform.* **5**:82–87.
16. Truncaite L, et al. 21 August 2012. Bacteriophage vB_EcoM_FV3: a new member of “rV5-like viruses.” *Arch. Virol.* doi:10.1007/s00705-012-1449-x.
17. Van Twest R, Kropinski AM. 2009. Bacteriophage enrichment from water and soil, p. 15–21. *In* Clokie MR, Kropinski AM (ed), *Bacteriophages: methods and protocols*, vol 1. Humana Press, New York, NY.
18. Zafar N, Mazumder R, Seto D. 2002. CoreGenes: a computational tool for identifying and cataloging “core” genes in a set of small genomes. *BMC Bioinformatics* **3**:12. doi:10.1186/1471-2105-3-12.