

# Complete Genome of Temperate Phage ENT39118 from *Cronobacter sakazakii*

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***Cronobacter sakazakii* infection is particularly harmful to infants, and putative virulence factors of prophage origin have been identified in *C. sakazakii*. In this study, the phage ENT39118 was isolated from wild-type *C. sakazakii*; it belongs to the family Siphoviridae. The genomic sequence of phage ENT39118 was composed of circular double-stranded DNA with a length of 39,012 bp. The sequence of ENT39118 showed weak sequence similarity to some reported regions of the prophage sequences in the *C. sakazakii* BAA-894 genome. To our knowledge, this is the first study of the genomic sequencing and annotation of this temperate phage, which was obtained from a *C. sakazakii* isolate from powdered infant formula.**

Infectious diseases caused by *Cronobacter sakazakii* are very important causes of meningitis and septicemia in neonates and infants (4, 12). Recently, Kucerova et al. reported the presence of putative prophage in the *C. sakazakii* genome in a study involving whole-genome analysis (7). Many bacteria become virulent after acquiring one or more prophages with toxins or other virulence factors (2, 3, 13). Therefore, it might be important to elucidate the relationship between *C. sakazakii* and its prophages. In this study, the genomic sequence of the temperate phage from wild-type *C. sakazakii* was determined to investigate the genomic organization of the phage.

Wild-type *C. sakazakii* was isolated from powdered infant formula and identified on the basis of its 16S rRNA sequence (8). To isolate the temperate phage from wild-type *C. sakazakii*, the bacterium was treated with mitomycin C. Morphological analysis using transmission electron microscopy showed that the phage, ENT39118, had a long, noncontractile tail and an icosahedral head and was therefore considered to belong to the family Siphoviridae (1).

The genome of phage ENT39118 was isolated using the method reported by Manfioletti and Schneider (10), and the genomic sequence was determined using ultra-high-throughput sequencing with a GS FLX Titanium genome sequencer. The genomic sequences were compared with those of the other genes in GenBank by using the BLAST (14). The open reading frames (ORFs) were determined with the NCBI ORF finder (14). The molecular weight and isoelectric point were calculated using the Compute pI/M<sub>w</sub> program (5). tRNAs were identified using the tRNAscan-SE programs (9). Conserved protein domain analysis was performed using BLASTP and the NCBI conserved domain database (CDD) (11).

The phage ENT39118 genome comprised circular double-stranded DNA and was 39,012 bp with 53.06% G+C content. A genome sequence search performed using BLASTN showed that the phage ENT39118 sequence had 29% and 39% sequence similarity with *C. sakazakii* BAA-894 (accession number CP000783) and *C. turicensis* z3032 (accession number FN543093), respectively. BLASTX and Prophinder analyses anticipated the presence of six putative prophage regions in the whole genome sequence of *C. sakazakii* BAA-894 (7). Dot plot analysis showed weak similarity among the various prophage sequences in *C. sakazakii* BAA-894. Interestingly, the genome sequence of phage ENT39118 showed weak sequence similarity to three regions of prophage I

and one region of prophage III in *C. sakazakii* BAA-894. Therefore, the genomic regions in *C. sakazakii* might be associated with rearrangements and mutations in the phage genome during lysogeny because of internal and external factors (6).

The ORFs from the genome were involved in replication, DNA packaging, morphogenesis, lysogeny, and lysis. The DNA packaging modules and morphogenesis modules contained a terminase, a phage portal protein, and head and tail morphogenesis-related proteins. In addition, the phage ENT39118 genome encoded lysogeny- and replication-related proteins, such as nuclease, primase, and integrase, and also encoded endolysin for host lysis. In conclusion, this study is the first report to show the morphological features and the complete genome sequence of the temperate phage in *C. sakazakii*.

**Nucleotide sequence accession number.** The complete genome sequence of temperate phage ENT39118 is available in GenBank under accession number [HQ201307](https://www.ncbi.nlm.nih.gov/nuclseq/HQ201307).

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