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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Cronobacter sakazakii* Bacteriophage CR3

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Due to the high risk of *Cronobacter sakazakii* infection in infants fed powdered milk formula and the emergence of antibiotic-resistant strains, an alternative biocontrol agent using bacteriophage is needed to control this pathogen. To further the development of such an agent, the *C. sakazakii*-targeting bacteriophage CR3 was isolated and its genome was completely sequenced. Here, we announce the genomic analysis results of the largest *C. sakazakii* phage known to date and report the major findings from the genome annotation.

Cronobacter sakazakii is an opportunistic food-borne pathogen that often contaminates powdered infant milk formula, vegetables, and fruits and causes septicemia, meningitis, and necrotizing enterocolitis in neonates (3, 6, 9). It has recently attracted public attention due to the extremely high risk to infants fed contaminated formula, with 50 to 80% fatality rates (5, 9). The high resistance of *C. sakazakii* to unusually dry conditions supports a high survival rate in powdered infant formula (4). However, the emergence of antibiotic-resistant *C. sakazakii* strains has limited the use of antibiotics to control this pathogen (12), suggesting that the development of alternative biocontrol agents, like bacteriophage, is urgently needed. Here, a novel *C. sakazakii* bacteriophage, CR3, was isolated from an environmental sample and its genome was completely sequenced.

Genomic DNA was isolated using a standard alkaline lysis method (10) and sequenced using GS-FLX Titanium by Macrogen, Seoul, South Korea. The quality-filtered reads were assembled using Newbler 2.3, and the prediction of open reading frames (ORFs) was performed using GeneMarkS (1), Glimmer3 (2), and FgenesV (Softberry, Inc., Mount Kisco, NY). Transfer RNAs were predicted using tRNAscan-SE (8), and conserved protein motif analyses of the predicted ORFs were conducted using InterProScan (11). Comparative codon preference analyses of the *C. sakazakii* BAA-894 (7) and phage CR3 genomes were carried out using CodonW 1.4.4 in the MOBYLE portal website (Pasteur Institute, Paris, France).

The complete genome of *C. sakazakii* phage CR3, belonging to the *Myoviridae* family, is 149,273 bp in length with a GC content of 50.95%, 265 ORFs, and 18 tRNAs, indicating the largest genome among *C. sakazakii* bacteriophages to date. Interestingly, this phage genome contains many tRNAs, and comparative codon preference analyses between the phage and *C. sakazakii* BAA-894 showed different codon preferences for valine, serine, alanine, lysine, asparagine, arginine, and glycine, suggesting that these extra phage tRNAs may play a role in the translation of phage mRNA, not host mRNA (data not shown). The genome of phage CR3 encodes structure/packaging proteins (major capsid protein, head stabilization/decoration protein, tail fiber proteins, tail fiber assembly protein, tape measure protein, and terminase), DNA manipulation proteins (DNA polymerases, DNA methylases, DNA primase, DNA helicase, DNA ligase, DNA methyltransferase, and endonucleases), and many additional functional proteins, such as a thymidylate synthase and a cell wall hydrolase, SleB. Although this genome encodes many ORFs, most of them are hypothetical proteins (84.5%), probably due to insufficient information about *C. sakazakii* phage genes in the GenBank database. Phage CR3 has two copies of tail fiber proteins targeting flagella of *C. sakazakii*, experimentally confirmed by the fact that CR3 did not infect the flagellum deletion mutant (data not shown). While this phage genome does not encode endolysin for host lysis, it encodes a cell wall hydrolase, SleB, suggesting that this protein may be involved in the host lysis. The complete genome analysis of *C. sakazakii* phage CR3 provides further information about *C. sakazakii* phages and extends the potential for application of phage CR3 as a natural biocontrol agent to control *C. sakazakii*.

Nucleotide sequence accession number. The complete genome sequence of *C. sakazakii* bacteriophage CR3 is available in GenBank under accession number JQ691612.

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