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GENOME ANNOUNCEMENT

Complete Genome Sequence of *Cronobacter sakazakii* Temperate Bacteriophage phiES15

Ju-Hoon Lee,[b] Younho Choi,[a] Hakdong Shin,[a] Junghyun Lee,[a] and Sangryeol Ryu[a]

Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul, South Korea,[a] and Department of Food Science and Biotechnology, Kyung Hee University, Yongin, South Korea[b]

While most phage genome studies have been focused on the virulent phages, the inducible temperate bacteriophage genome study provides more detailed information about the interaction between the host strain and the phage. To study this interaction in detail, UV-induced phiES15 bacteriophage was isolated from the host strain *Cronobacter sakazakii* ES15 and its genome was completely sequenced. Here we announce the genome sequence of phiES15 and report major findings from the annotation.

Cronobacter sakazakii has been recognized as a critical pathogenic bacterium, especially for powdered formula-fed infants, due to high (up to 80%) mortality (6, 7, 12). While various antibiotics have been used to control this pathogen, recent studies reported emergence of antibiotic-resistant strains (14). Therefore, bacteriophage therapy using *C. sakazakii*-targeting phages has been suggested as an alternative treatment to control this pathogen (8, 15). To optimize this phage therapy, a study of the molecular interaction between the *C. sakazakii* host and its specific phages needs to be done. Furthermore, the study of the interaction between the *C. sakazakii* host and its temperate phage would provide information in greater detail than studies of virulent phages targeting *C. sakazakii*. To study the details of interaction, prophage-harboring *C. sakazakii* ES15 was isolated from the powder of ground whole grains and its temperate phage, designated phiES15, was also isolated from the host strain after UV induction. Here the genome of phage phiES15 was completely sequenced and analyzed.

Isolated and purified genomic DNA of phiES15 was sequenced using a GS-FLX 454 pyrosequencer (Macrogen, Seoul, South Korea), and the qualified reads were assembled using GS De Novo Assembler 2.3. The predictions of open reading frames (ORFs) were conducted using three major gene prediction programs, GeneMarkS (2), Glimmer3 (5), and FgenesB (Softberry, Inc., Mount Kisco, NY) and confirmed by ribosomal binding site analyses performed using RBS finder (J. Craig Venter Institute, Rockville, MD). The functions of ORFs were predicted by BLASTP analyses using the Conserved Domain Database (CDD) (1, 10) and conserved protein motif analyses using InterProScan (13). tRNA was predicted by the use of tRNAscan-SE software (9), and the annotated genome information was handled using Artemis14 (3).

The phiES15 genome has 39,974 bp containing 52 ORFs and no tRNA, with GC content of 53.54%. While 23 (43.3%) ORFs among them were hypothetical proteins, other ORFs were predicted to be functional, and they were categorized into seven groups: integration (integrase and excisionase), DNA modification and recombination (adenine-specific DNA methyltransferase and crossover junction endodeoxyribonuclease RusA), host interaction (host-nuclease inhibitor protein Gam and host division inhibitor protein Kil), transcription regulation (transcriptional repressor DicA, CII, and antitermination protein Q), replication (replication proteins O and P), host lysis (endolysin and Rz/Rz1), and phage packaging (terminase small and large subunits). It is intriguing that the phiES15 genome encodes host interaction proteins such as Gam (probably involved in protection of phiES15 phage genome from the foreign DNA degradation by host nucleases) (11) and Kil (probably involved in utilization of the host resources for phage reconstruction by inhibition of the host cell division) (4). In addition, a 26-bp *attP* sequence is shared between the phage phiES15 and the host strain ES15, substantiating the hypothesis that this phiES15 is a temperate phage and that it serves as a site of phage integration into the host genome. The study of the complete genome of the phiES15 phage would provide further information about the interaction between *C. sakazakii* host and its temperate phage.

Nucleotide sequence accession number. The complete genome sequence of *Cronobacter sakazakii* phage phiES15 is available in GenBank under accession number JQ780327.

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REFERENCES

1. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ.** 1990. Basic local alignment search tool. J. Mol. Biol. **215**:403–410.
2. **Besemer J, Lomsadze A, Borodovsky M.** 2001. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. **29**:2607–2618.
3. **Carver T, et al.** 2008. Artemis and ACT: viewing, annotating and comparing sequences stored in a relational database. Bioinformatics **24**:2672–2676.
4. **Conter A, Bouché JP, Dassain M.** 1996. Identification of a new inhibitor of essential division gene *ftsZ* as the *kil* gene of defective prophage Rac. J. Bacteriol. **178**:5100–5104.
5. **Delcher AL, Bratke KA, Powers EC, Salzberg SL.** 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. Bioinformatics **23**:673–679.

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Address correspondence to Sangryeol Ryu, sangryu@snu.ac.kr.

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6. **Drudy D, Mullane NR, Quinn T, Wall PG, Fanning S.** 2006. *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. Clin. Infect. Dis. **42**:996–1002.
7. **Healy B, et al.** 2010. *Cronobacter* (*Enterobacter sakazakii*): an opportunistic foodborne pathogen. Foodborne Pathog. Dis. **7**:339–350.
8. **Kim K-P, Klumpp J, Loessner MJ.** 2007. *Enterobacter sakazakii* bacteriophages can prevent bacterial growth in reconstituted infant formula. Int. J. Food Microbiol. **115**:195–203.
9. **Lowe TM, Eddy SR.** 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. **25**: 955–964.
10. **Marchler-Bauer A, et al.** 2011. CDD: a Conserved Domain Database for the functional annotation of proteins. Nucleic Acids Res. **39**(Database issue):D225–D229.
11. **Marsić N, Roje S, Stojiljković I, Salaj-Smic E, Trgovcević Z.** 1993. In vivo studies on the interaction of RecBCD enzyme and lambda Gam protein. J. Bacteriol. **175**:4738–4743.
12. **Nazarowec-White M, Farber JM.** 1997. *Enterobacter sakazakii*: a review. Int. J. Food Microbiol. **34**:103–113.
13. **Zdobnov EM, Apweiler R.** 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. Bioinformatics **17**: 847–848.
14. **Zhou X, Gao J, Huang Y, Fu S, Chen H.** 2011. Antibiotic resistance pattern of *Klebsiella pneumoniae* and *Enterobacter sakazakii* isolates from powdered infant formula. Afr. J. Microbiol. Res. **5**:3073–3077.
15. **Zuber S, et al.** 2008. Decreasing *Enterobacter sakazakii* (*Cronobacter* spp.) food contamination level with bacteriophages: prospects and problems. Microb. Biotechnol. **1**:532–543.