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J. Bacteriol. 2012, 194(16):4438. DOI: 10.1128/JB.00841-12.

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Complete Genome Sequence of the Opportunistic Food-Borne Pathogen *Cronobacter sakazakii* ES15

Hakdong Shin,^a Ju-Hoon Lee,^b Younho Choi,^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

***Cronobacter sakazakii* is an emerging pathogen associated with several outbreaks of food-borne illness in premature infants. To characterize its physiology and pathogenicity at the molecular level, *C. sakazakii* ES15 was isolated and its genome was completely sequenced and analyzed. Here, the results are announced and major findings from its annotation data are reported.**

Cronobacter sakazakii is a Gram-negative opportunistic food-borne pathogen especially contaminating powdered milk formula for infants (4, 9). Recently, it has come into the spotlight due to the high risk to powdered-formula-fed infants, with 50 to 80% mortality (6). Interestingly, its production of capsular material was reported (7), suggesting that this capsule formation may contribute to its high survival rate in extremely dry conditions. In addition, it causes meningitis, bacteremia, and necrotizing enterocolitis in infants, probably due to its effective invasion into intestinal epithelial cells and brain microvascular endothelial cells (BMEC) (15). To further understand the physiology and pathogenicity of this pathogen at the molecular level, its genome was completely sequenced and analyzed.

C. sakazakii ES15 was originally isolated from ground whole grains, and the genomic DNA was sequenced using a GS-FLX pyrosequencer (Macrogen, South Korea). Prediction of the open reading frames (ORFs) was first performed using GeneMarkS (2) and Glimmer3 (3). The functional analyses of ORFs were conducted using BLASTP and InterProScan (1, 17). Transfer RNAs and CRISPR repeat regions were predicted using tRNAscan-SE (13) and CRISPR finder (5). The functional categorization and metabolic pathway analyses, respectively, were carried out using the COG and KEGG databases (10, 16).

The complete genome of *C. sakazakii* ES15 revealed 4,268,675 bp containing 3,916 ORFs, 7 rRNA operons, and 80 tRNAs with a GC content of 57.11%. In addition, this genome has two prophages and two CRISPR loci containing 9 and 16 CRISPR repeats, respectively. Interestingly, one of the prophages, phiES15, is UV inducible, and its genome sequence was recently analyzed to elucidate the interaction between the host strain and this phage. The metabolic/biosynthetic pathway analysis using the KEGG database showed that this genome has complete sets of genes for glycolysis and the tricarboxylic acid (TCA) cycle, as well as for flagellum assembly, substantiating the idea that this bacterium is really facultative aerobic and motile (8). In addition, it also has essential genes for biosynthesis of 20 amino acids. However, two aminoacyl-tRNA synthetases, the glutamyl-tRNA and asparaginyl-tRNA synthetases, are missing, suggesting that *C. sakazakii* may have alternative routes for successful translations of glutamine and asparagine (14). Interestingly, this genome has a relatively high number of ABC transport systems and phosphotransferase systems (PTS), suggesting that *C. sakazakii* has efficient nutrient uptake systems. It is intriguing that the *C. sakazakii* ES15 genome encodes an outer membrane protein A (OmpA; ES15_2832),

which is probably involved in its invasion into BMEC, suggesting its pathogenicity (11). However, IbeB, a component of the copper/silver resistance cation efflux system, was not detected in this genome, which is different from *C. sakazakii* BAA-894 (12). While the complete genome sequence analysis of *C. sakazakii* increases our knowledge of the characteristics of this pathogenic bacterium in the extremely dry condition, further study of its pathogenicity at the molecular level needs to be elucidated with the help of this complete genome annotation.

Nucleotide sequence accession number. The complete genome sequence of *Cronobacter sakazakii* ES15 is available in GenBank under the accession number CP003312.

ACKNOWLEDGMENT

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the government of South Korea (MEST) (grant no. 2010-0026030).

REFERENCES

- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.
- 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.
- Delcher AL, Bratke KA, Powers EC, Salzberg SL. 2007. Identifying bacterial genes and endosymbiont DNA with Glimmer. *Bioinformatics* 23:673–679.
- 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.
- Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res.* 35:W52–W57.
- Healy B, et al. 2010. *Cronobacter (Enterobacter sakazakii)*: an opportunistic foodborne pathogen. *Foodborne Pathog. Dis.* 7:339–350.
- Hurrell E, Kucerova E, Loughlin M, Caubilla-Barron J, Forsythe SJ. 2009. Biofilm formation on enteral feeding tubes by *Cronobacter sakazakii*, *Salmonella* serovars and other *Enterobacteriaceae*. *Int. J. Food Microbiol.* 136:227–231.

Received 14 May 2012 Accepted 31 May 2012

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

H.S. and J.-H.L. contributed equally to this work.

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doi:10.1128/JB.00841-12

8. Iversen C, et al. 2007. The taxonomy of *Enterobacter sakazakii*: proposal of a new genus *Cronobacter* gen. nov. and descriptions of *Cronobacter sakazakii* comb. nov., *Cronobacter sakazakii* subsp. *sakazakii*, comb. nov., *Cronobacter sakazakii* subsp. *malonaticus* subsp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov. and *Cronobacter genomospecies* 1. BMC Evol. Biol. 7:64. doi:10.1186/1471-2148-7-64.
9. Kandhai MC, Reij MW, Gorris LGM, Guillaume-Gentil O, van Schothorst M. 2004. Occurrence of *Enterobacter sakazakii* in food production environments and households. Lancet 363:39–40.
10. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. 2012. KEGG for integration and interpretation of large-scale molecular data sets. Nucleic Acids Res. 40:D109–D114.
11. Kim K, et al. 2010. S. Outer membrane protein A (OmpA) and X (OmpX) are essential for basolateral invasion of *Cronobacter sakazakii*. Appl. Environ. Microbiol. 76:5188–5198.
12. Kucerova E, et al. 2010. Genome sequence of *Cronobacter sakazakii* BAA-894 and comparative genomic hybridization analysis with other *Cronobacter* species. PLoS One 5:e9556. doi:10.1371/journal.pone.0009556.
13. 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.
14. Rogers KC, Soll D. 1995. Divergence of glutamate and glutamine aminoacylation pathways: providing the evolutionary rationale for mischarging. J. Mol. Evol. 40:476–481.
15. Singamsetty VK, Wang Y, Shimada H, Prasadarao NV. 2008. Outer membrane protein A expression in *Enterobacter sakazakii* is required to induce microtubule condensation in human brain microvascular endothelial cells for invasion. Microb. Pathog. 45:181–191.
16. Tatusov R, et al. 2003. The COG database: an updated version includes eukaryotes. BMC Bioinform. 4:41. doi:10.1186/1471-2105-4-41.
17. Zdobnov EM, Apweiler R. 2001. InterProScan—an integration platform for the signature-recognition methods in InterPro. Bioinformatics 17: 847–848.