

Complete Genome Sequence of *Cronobacter sakazakii* Strain CMCC 45402

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Cronobacter sakazakii is considered to be an important pathogen involved in life-threatening neonatal infections. Here, we report the annotated complete genome sequence of *C. sakazakii* strain CMCC 45402, obtained from a milk sample in China. The major findings from the genomic analysis provide a better understanding of the isolates from China.

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ronobacter spp. (formerly Enterobacter sakazakii) are Gramnegative opportunistic pathogens that cause life-threatening infections, such as meningitis, sepsis, bacteremia, and necrotizing enterocolitis in neonates, children, and immune compromised adults, particularly older persons (1, 2). Cases of meningitis and necrotizing enterocolitis following Cronobacter sakazakii infection both have high mortality rates of 40 to 80% and 10 to 55%, respectively (3, 4). Cronobacter spp. have been recovered from a wide variety of foods, including powdered infant formula (PIF), weaning foods, meat, cheese, vegetables, grains, herbs, spices, milk, teas, dried infant and adult cereals, tofu, chocolate, and pastas, from environments, such as factories and households, and even from insects (5-7). Several studies have shown the prevalence of C. sakazakii in dairy products in China to be from 1.22 to ~66.67% (8). These organisms are known for their stress response. Until the present, little has been known about the mechanisms of pathogenicity in Cronobacter species. In the present study, C. sakazakii CMCC 45402 was isolated from milk samples in China. To enhance our understanding of this microorganism, wholegenome and plasmid sequencing were performed.

Whole-genome sequencing of C. sakazakii CMCC 45402, which was isolated from the milk samples in China, was performed with a combined strategy using a 454 sequencing and Solexa paired-end sequencing technology. Genomic libraries containing 8-kb inserts were constructed, and 269,508 paired-end reads and 94,334 single-end reads were generated using the 454 GS FLX+ system, giving 24.7-fold coverage of the genome. Automatic assembly was done using the Newbler 2.6 software (454 Life Sciences, Branford, CT) and yielded 6 large scaffolds, including 46 nonredundant contigs. A total of 8,292,276 paired-end reads (500-bp library) were generated to reach a depth of 359-fold coverage with the Illumina genome analyzer IIx (Illumina, San Diego, CA), and they were mapped to the scaffolds using the Burrows-Wheeler Aligner (BWA). Sequence gaps were filled through the sequencing of PCR products generated from an ABI 3730. Prediction and annotation of protein-encoding genes were performed as described previously.

The complete genome sequence of *C. sakazakii* CMCC 45402 contains a circular 4,377,544-bp chromosome with a G+C content of 56.89% and two circular plasmids of 126,488 bp and 55,913 bp.

In total, there are 4,249 predicted genes in the chromosome, including 4,160 protein-encoding genes, 82 tRNA-encoding genes, and 7 rRNA-encoding genes. Of the 4,160 predicted protein-encoding genes, biological roles were assigned to 2,658 genes (63.89%) with similarity to proteins of known function according to the COG scheme. Of the total, 1,747 (42%) predicted coding sequences matched gene products of unknown function from other species, and 156 (3.6%) had no database match.

Five hundred sixty (13.5%) genes of the predicted proteinencoding genes are involved in metabolic pathways, and 253 (6.1%) participate in the biosynthesis of secondary metabolites. These predicted genes also participate in pathways, such as microbial metabolism in diverse environments, purine metabolism, and the ABC transporter pathway.

Nucleotide sequence accession numbers. The sequence and annotation of the *C. sakazakii* CMCC 45402 genome have been deposited in GenBank under the accession no. CP006731, accompanied by two plasmid sequences (accession no. CP006732 and CP006733).

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REFERENCES

- Gosney MA, Martin MV, Wright AE, Gallagher M. 2006. Enterobacter sakazakii in the mouths of stroke patients and its association with aspiration pneumonia. Eur. J. Intern. Med. 17:185–188. http://dx.doi.org/10.1016/j .ejim.2005.11.010.
- Skovgaard N. 2007. New trends in emerging pathogens. Int. J. Food Microbiol. 120:217–224. http://dx.doi.org/10.1016/j.ijfoodmicro.2007.07.046.
- 3. Muytjens HL, Zanen HC, Sonderkamp HJ, Kollée LA, Wachsmuth IK, Farmer JJ III. 1983. Analysis of eight cases of neonatal meningitis and sepsis due to *Enterobacter sakazakii*. J. Clin. Microbiol. **18**:115–120.

- Forsythe SJ. 2005. Enterobacter sakazakii and other bacteria in powdered infant milk formula. Matern. Child Nutr. 1:44–50. http://dx.doi.org/10.11 11/j.1740-8709.2004.00008.x.
- Drudy D, O'Rourke M, Murphy M, Mullane NR, O'Mahony R, Kelly L, Fischer M, Sanjaq S, Shannon P, Wall P, O'Mahony M, Whyte P, Fanning S. 2006. Characterization of a collection of *Enterobacter sakazakii* isolates from environmental and food sources. Int. J. Food Microbiol. 110: 127–134. http://dx.doi.org/10.1016/j.ijfoodmicro.2006.02.008.
- 6. Nazarowec-White M, Farber JM. 1997. Thermal resistance of Enterobacter

sakazakii in reconstituted dried-infant formula. Lett. Appl. Microbiol. 24: 9–13. http://dx.doi.org/10.1046/j.1472-765X.1997.00328.x.

- Baumgartner A, Grand M, Liniger M, Iversen C. 2009. Detection and frequency of *Cronobacter spp.* (*Enterobacter sakazakii*) in different categories of ready-to-eat foods other than infant formula. Int. J. Food Microbiol. 136:189–192. http://dx.doi.org/10.1016/j.ijfoodmicro.2009. 04.009.
- 8. Xiaoling Q, Guopu R. 2011. Research advance of *E. sakazakii* in dairy products. China Dairy Ind. **39:**45–46, 49.