

# Complete Genome Sequence of *Cronobacter turicensis* LMG 23827, a Food-Borne Pathogen Causing Deaths in Neonates<sup>∇</sup>

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**Here, we report the complete and annotated genome sequence of *Cronobacter turicensis*, an opportunistic food-borne pathogen, which is known as a rare but important cause of life-threatening neonatal infections. Among all proteins of *C. turicensis*, 223 have been annotated as virulence- and disease-related proteins.**

*Cronobacter* spp., previously known as *Enterobacter sakazakii*, are Gram-negative opportunistic food-borne pathogens and are known as rare but important causes of life-threatening neonatal infections which can lead to severe disease manifestations, such as brain abscesses, meningitis, necrotizing enterocolitis, and systemic sepsis (6). Neonates and infants under 2 months of age who were born prematurely are at greater risk of *Cronobacter* infections from consuming *Cronobacter*-contaminated powdered infant formulas (2). Updating the original taxonomy of *E. sakazakii* by using a polyphasic approach has resulted in the definition of six new species based on extensive geno- and phenotypic evaluations, *Cronobacter sakazakii*, *Cronobacter malonaticus*, *Cronobacter muytjensii*, *Cronobacter dublinensis*, *Cronobacter turicensis*, and *Cronobacter* genomospecies 1 (3). The definition of six species within the genus *Cronobacter* is further supported by the results of recent studies in which a multilocus sequence analysis (MLSA) and a matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) mass spectrometry approach were used (5, 10).

We are mainly interested in the virulence factors of this opportunistic food-borne pathogen. Until the present, little has been known about the mechanisms of pathogenicity in *Cronobacter* spp. Few studies have described aspects of the interaction of *Cronobacter* spp. with human cells (4, 7, 8, 9). Several putative virulence factors were recently described in *Cronobacter turicensis* using a proteomic approach (1).

In the present study, *Cronobacter turicensis* LMG 23827, a strain that caused the deaths of two newborn children in a children's hospital in Zürich in 2005, was chosen to sequence and annotate the genome of a representative and evidently pathogenic member of the genus *Cronobacter*. Whole-genome sequencing was done using the combination of a fosmid-sequencing approach (Sanger) and 454 pyrosequencing (GATC, Konstanz, Germany).

The genome of *C. turicensis* consists of a circular chromosome with a size of 4,384,526 bp and three plasmids with sizes

of 138,339 bp, 22,448 bp, and 53,842 bp. Altogether, 4,455 coding sequences were identified, of which 9.27% ( $n = 413$ ) did not show similarities to other proteins in public sequence databases and therefore remain unknown proteins. The genome encodes 84 tRNAs with 40 different codons for 21 amino acids, including selenocysteine. Seven rRNA operons could be found in the genome, which is comparable to many other *Enterobacteriaceae*. The remarkable number of 122 coding sequences has been predicted as putative pseudogenes. Almost 95% of these probably untranscribed genes retained detectable homology to annotated genes in other organisms, allowing investigation into which particular functions are putative targets of gene degradation.

Among all proteins of *C. turicensis*, 22 show strong homology to proteins annotated with the UniProtKB/Swiss-Prot (11) keyword “virulence” and 223 have been annotated to as virulence- and disease-related proteins. Specific virulence determinants in *C. turicensis* comprise a putative O-antigen gene cluster, putative genes for the acquisition of iron and the production of slime, for capsular polysaccharides, and for enterobacterial common antigen, and genes encoding a flagellum apparatus, pili, a probably functional type IV secretion system, and a SecA-dependent pathway, as well as effector proteins.

**Nucleotide sequence accession numbers.** The nucleotide sequences are available under RefSeq accession numbers NC\_013282 to NC\_013285 and GenBank accession numbers FN543093 to FN543096.

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## REFERENCES

1. Carranza, P., I. Hartmann, A. Lehner, R. Stephan, P. Gehrig, J. Grossmann, S. Barkow-Oesterreicher, B. Roschitzki, L. Eberl, and K. Riedel. 2009. Proteomic profiling of *Cronobacter turicensis* 3032, a food-borne opportunistic pathogen. *Proteomics* 9:3564–3579.
2. Hunter, C. J., M. Petrosyan, H. R. Ford, and N. V. Prasadarao. 2008. *Enterobacter sakazakii*: an emerging pathogen in infants and neonates. *Surg. Infect.* 9:533–539.
3. Iversen, C., N. Mullane, B. McCardell, B. D. Tall, A. Lehner, S. Fanning, R. Stephan, and H. Joosten. 2008. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov. comb. nov., *C. malonaticus* sp. nov., *C. turicensis* sp. nov., *C. muytjensii* sp. nov., *C. dublinensis* sp. nov., *Cronobacter*

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- genomospecies 1, and of three subspecies, *C. dublinensis* sp. nov. subsp. *dublinensis* subsp. nov., *C. dublinensis* sp. nov. subsp. *lausannensis* subsp. nov., and *C. dublinensis* sp. nov. subsp. *lactaridi* subsp. nov. Int. J. Syst. Evol. Microbiol. **58**:1442–1447.
4. **Kim, K. P., and M. J. Loessner.** 2008. Enterobacter sakazakii invasion in human intestinal Caco-2 cells requires the host cell cytoskeleton and is enhanced by disruption of tight junction. Infect. Immun. **76**:562–570.
  5. **Kuhnert, P., B. M. Korczak, R. Stephan, H. Joosten, and C. Iversen.** 2009. Phylogeny and whole genome DNA-DNA similarity of *Cronobacter* (*Enterobacter sakazakii*) and related species by multilocus sequence analysis (MLSA). Int. J. Food Microbiol. **136**:152–158.
  6. **Lehner, A., and R. Stephan.** 2004. Microbiological, epidemiological and food safety aspects of *Enterobacter sakazakii*. J. Food Prot. **67**:2850–2857.
  7. **Mange, J. P., R. Stephan, N. Borel, P. Wild, K. S. Kim, A. Pospischil, and A. Lehner.** 2006. Adhesive properties of *Enterobacter sakazakii* to human epithelial and brain microvascular endothelial cells. BMC Microbiol. **6**:58.
  8. **Mohan Nair, M. K., and K. Venkitanarayanan.** 2007. Role of bacterial OmpA and host cytoskeleton in the invasion of human intestinal epithelial cells by *Enterobacter sakazakii*. Pediatr. Res. **62**:664–669.
  9. **Pagotto, F. J., M. Nazarowec-White, S. Bidawid, and J. M. Farber.** 2003. *Enterobacter sakazakii*: infectivity and enterotoxin production in vitro and in vivo. J. Food Prot. **66**:370–375.
  10. **Stephan, R., D. Ziegler, V. Pflüger, G. Vogel, and A. Lehner.** 2010. Rapid genus- and species-specific identification of *Cronobacter* spp. by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J. Clin. Microbiol. **48**:2846–2851.
  11. **UniProt Consortium.** 2009. The Universal Protein Resource (UniProt) 2009. Nucleic Acids Res. **37**:D169–D174.