



Genome Sequence of *Cronobacter sakazakii* Serogroup O:4, Sequence Type 4 Strain CDC 2009-03746, Isolated from a Fatal Case of Infantile Meningitis

Christopher J. Grim, Gopal R. Gopinath, Karen G. Jarvis, Venugopal Sathyamoorthy, Larissa H. Trach, Hannah R. Chase, Ben D. Tall Center for Food Safety and Applied Nutrition, U. S. Food and Drug Administration, Laurel, Maryland, USA

We report the draft genome sequence of a *Cronobacter sakazakii* serogroup O:4, sequence type 4 strain, CDC 2009-03746 (=NM1240=2009-06-01), isolated from a fatal case of infantile meningitis. The draft genome has a size of 4,492,904 bp and a G+C% content of 56.7.

Received 9 April 2015 Accepted 23 April 2015 Published 21 May 2015

Citation Grim CJ, Gopinath GR, Jarvis KG, Sathyamoorthy V, Trach LH, Chase HR, Tall BD. 2015. Genome sequence of *Cronobacter sakazakii* serogroup O:4, sequence type 4 strain CDC 2009-03746, isolated from a fatal case of infantile meningitis. Genome Announc 3(3):e00492-15. doi:10.1128/genomeA.00492-15.

Copyright © 2015 Grim et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 3.0 Unported license.

Address correspondence to Christopher J. Grim, christopher.grim@fda.hhs.gov.

C*ronobacter* comprise a diverse genus of pathogens that cause life-threatening infantile infections, such as meningitis, necrotizing enterocolitis, and septicemia (1, 2). Baumbach et al. (3) recently reported the isolation of three *Cronobacter sakazakii* strains, from two infant cases, which belong to the newly described *C. sakazakii* O:4 molecular serotype (4). One of the three isolates, *C. sakazakii* strain 2009-03746 (=NM1240=2009-06-01), also belongs to sequence type 4 (ST4; http://pubmlst.org/cronobacter), which is implicated as a major cause of neonatal infections (5). To date, clinical ST4 strains which have been sequenced have been shown to belong to the Csak O:2 molecular serotype (6–8).

To further understand this isolate, whole-genome sequencing was performed on the MiSeq platform (Illumina, San Diego, CA, USA), utilizing 500 cycles of paired-end reads (Illumina). Fastq datasets were *de novo* assembled with CLC Genomics Workbench version 7.0 (CLC bio, Aarhus, Denmark). The draft genome of 2009-03746 is 4,492,904 bp, on 128 contigs (>200 bp in size) and has a G+C% content of 56.7. Genomic contigs were annotated using the RAST annotation server (9), and predicted to contain 4,170 coding sequences.

Interestingly, the genome of C. sakazakii 2009-03746 has a high nucleotide identity (average nucleotide identity [ANI] of 99.8%) with members of the clonal C. sakazakii O:2 group (10), such as C. sakazakii 2151 and C. sakazakii ES713 (GenBank accession numbers AJKT00000000.1 and AJLB00000000.1, respectively). In comparison, the ANI of 2009-03746 with another Csak O:4 isolate, C. sakazakii E764 (GenBank accession number AJLA00000000.1), is only 98.0%. The overall nucleotide identity with Csak O:2 genomes would be higher, except for the presence of an apparent recombination event(s) involving the 171-kb region located between tRNA-Proline-GGG and tRNA-Asparagine-GTT₁. This region contains genes involved in extracellular polysaccharide synthesis, including colanic acid and O-antigen biosynthesis, putative galactose and mannose LPS modification operons, and the yeh osmoprotectant operon. Surprisingly, in C. sakazakii strain 2009-03746, the majority of this region has high nucleotide identity with

that of *C. sakazakii* ATCC BAA-894, a Csak O:1 ST1 isolate, except for the Csak O:4 O-antigen region.

In terms of genome content, *C. sakazakii* strain 2009-03746 possesses many of the features of the species, as determined previously (11). Of note, this strain possesses genes for nine chaperone-usher fimbriae, cellulose synthesis, and sialic acid utilization operons; a 120-kb pESA3-like virulence plasmid (12); and a CRISPR element. The genome contains an additional 52-kb plasmid, homologous to plasmid pSP291-2 of *C. sakazakii* strain SP291 (GenBank accession number CP004093.1, ST4, Csak O:2) (7), which encodes copper homeostasis and heavy metal resistance operons.

Pathogen-specific virulence factors have been discovered on a variety of mobile genetic elements, such as plasmids and transposons. The "genomic plasticity" demonstrated by *C. sakazakii* strain 2009-03746 suggests ongoing genomic rearrangement, and these recombination events will undoubtedly complicate our efforts to classify these organisms into sharply delineated genomopathotypes.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JZDO000000000. The version described in this paper is version JZDO00000000.1

ACKNOWLEDGMENTS

We report no financial or other conflicts of interest relevant to the subject of this article.

C.J.G. was supported by an Oak Ridge Institute for Science and Education fellowship award. L.H.T. and H.R.C. were supported through two Joint Institute for Food Safety and Applied Nutrition student internship program awards.

REFERENCES

 Iversen C, Mullane N, McCardell B, Tall BD, Lehner A, Fanning S, Stephan R, Joosten H. 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., Crono*bacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. nov., *Cronobacter. dublinensis* subsp. *lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. Int J Syst Evol Microbiol **58**:1442–1447.

- Joseph S, Cetinkayaz E, Drahovska H, Levican A, Figueras MJ, Forsythe SJ. 2012. Cronobacter condimenti sp. nov., isolated from spiced meat, and Cronobacter unversalis sp. nov., a species designation for Cronobacter sp. genomospecies 1, recovered from a leg infection, water and food ingredients. Int. J Syst Evol Microbiology 62:1277–1283. http://dx.doi.org/ 10.1099/ijs.0.032292-0.
- Centers for Disease Control and Prevention (CDC). 2009. Cronobacter species isolation in two infants—New Mexico, 2008. MMWR Morb Mortal Wkly Rep 58:1179–1183.
- Jarvis KG, Yan QQ, Grim CJ, Power KA, Franco AA, Hu L, Gopinath G, Sathyamoorthy V, Kotewicz ML, Kothary MH, Lee C, Sadowski J, Fanning S, Tall BD. 2013. Identification and characterization of five new molecular serogroups of *Cronobacter* spp. Foodborne Pathog Dis 10: 343–352. http://dx.doi.org/10.1089/fpd.2012.1344.
- Hariri S, Joseph S, Forsythe SJ. 2013. Cronobacter sakazakii ST4 strains and neonatal meningitis, United States. Emerg Infect Dis 19:175–177. http://dx.doi.org/10.3201/eid1901.120649.
- Masood N, Moore K, Farbos A, Hariri S, Paszkiewicz K, Dickins B, McNally A, Forsythe S. 2013. Draft genome sequence of the earliest *Cronobacter sakazakii* sequence type 4 strain, NCIMB 8272. Genome Announc 1(5):e00782-13. http://dx.doi.org/10.1128/genomeA.00782-13.
- 7. Power KA, Yan Q, Fox EM, Cooney S, Fanning S. 2013. Genome sequence of *Cronobacter sakazakii* SP291, a persistent thermotolerant iso-

late derived from a factory producing powdered infant formula. Genome Announc 1(2):e00082-13. http://dx.doi.org/10.1128/genomeA.00082-13.

- Masood N, Moore K, Farbos A, Hariri S, Block C, Paszkiewicz K, Dickins B, McNally A, Forsythe S. 2013. Draft genome sequence of a meningitic isolate of *Cronobacter sakazakii* clonal complex 4, strain 8399. Genome Announc 1(5): e00833-13. http://dx.doi.org/10.1128/genomeA.00833-13.
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. http://dx.doi.org/10.1186/ 1471-2164-9-75.
- Mullane N, O'Gaora P, Nally JE, Iversen C, Whyte P, Wall PG, Fanning S. 2008. Molecular analysis of the *Enterobacter sakazakii* O-antigen gene locus. Appl Environ Microbiol 74:3783–3794. http://dx.doi.org/10.1128/ AEM.02302-07.
- Grim CJ, Kotewicz ML, Power KA, Gopinath G, Franco AA, Jarvis KG, Yan QQ, Jackson SA, Sathyamoorthy V, Hu L, Pagotto F, Iversen C, Lehner A, Stephan R, Fanning S, Tall BD. 2013. Pan-genome analysis of the emerging foodborne pathogen *Cronobacter* spp. suggests a specieslevel bidirectional divergence driven by niche adaptation. BMC Genomics 14:366. http://dx.doi.org/10.1186/1471-2164-14-366.
- Franco AA, Hu L, Grim CJ, Gopinath G, Sathyamoorthy V, Jarvis KG, Lee C, Sadowski J, Kim J, Kothary MH, McCardell BA, Tall BD. 2011. Characterization of putative virulence genes on the related RepFIB plasmids harbored by *Cronobacter* spp. Appl Environ Microbiol 77: 3255–3267. http://dx.doi.org/10.1128/AEM.03023-10.