AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Fully Closed Genome Sequences of Five Type Strains of the Genus *Cronobacter* and One *Cronobacter sakazakii* Strain

Deborah Moine,[a] Mohamed Kassam,[a] Leen Baert,[b] Yanjie Tang,[b] Caroline Barretto,[b] Catherine Ngom Bru,[b] Adrianne Klijn,[b] Patrick Descombes[a]

Nestlé Institute of Health Sciences SA, EPFL Innovation Park, Lausanne, Switzerland[a]; Nestec Ltd., Nestle Research Center, Lausanne, Switzerland[b]

***Cronobacter* is associated with infant infections and the consumption of reconstituted infant formula. Here we sequenced and closed six genomes of *C. condimenti*^{T}, *C. muytjensii*^{T}, *C. universalis*^{T}, *C. malonaticus*^{T}, *C. dublinensis*^{T}, and *C. sakazakii* that can be used as reference genomes in single nucleotide polymorphism (SNP)-based next-generation sequencing (NGS) analysis for source tracking investigations.**

Received 8 February 2016 **Accepted** 9 February 2016 **Published** 24 March 2016

Citation Moine D, Kassam M, Baert L, Tang Y, Barretto C, Ngom Bru C, Klijn A, Descombes P. 2016. Fully closed genome sequences of five type strains of the genus *Cronobacter* and one *Cronobacter sakazakii* strain. Genome Announc 4(2):e00142-16. doi:10.1128/genomeA.00142-16.

Address correspondence to Deborah Moine, Deborah.Moine@rd.nestle.com.

Cronobacter (formerly *Enterobacter sakazakii*) is a foodborne pathogen that has been identified as the causative agent of severe clinical complications in neonates and infants, such as meningitis, necrotizing enterocolitis, and septicemia (1, 2). The origin of this pathogen is not clear, but *Cronobacter* has been isolated from a wide range of foods, among which powdered infant formula (PIF) has been identified as the dominant vehicle of transmission (3, 4). *Cronobacter* is also often isolated from the environment and can be found in soil samples, domestic kitchens, and predominantly PIF manufacturing facilities (5, 6). The genus *Cronobacter* represents *E. sakazakii*, which was reclassified in 2007 as a result of biotyping and genotyping studies (7, 8).

Reliable identification and discrimination of *Cronobacter* strains is of importance due to the severe illness and ubiquitous occurrence in the environment and food. A multilocus sequence typing (MLST) scheme (9) has been shown to enable differentiation of closely related *Cronobacter* strains. The high discriminatory power and the drop in the cost of the next-generation sequencing (NGS) technologies favor the use of NGS as a routine diagnostic tool in public health reference laboratories in the near future (10). Clustering of *Cronobacter* isolates based on NGS data will allow a powerful source-tracking analysis. The clustering and the creation of phylogenic trees based on single nucleotide polymorphism (SNP) analysis of the NGS data are carried out by mapping short read sequences of *Cronobacter* isolates to a reference genome. The identification of reference genomes is essential for a reliable SNP-based analysis. Only a few complete genomes of *Cronobater* are available in public databases and the pool of reference genomes needs to be extended. Therefore, an effort was done to sequence and close genomes of *Cronobacter* spp. that can be used in SNP-based NGS analysis to support detailed source tracking investigations.

Genomic DNA was extracted from midexponential cultures using a Gentra DNA Purgene kit (Qiagen), and then 20-kb libraries were prepared following Pacific Biosciences (PacBio) protocol and Blupippin size selection. Sequencing was performed on the PacBio RSII platform using P4/C2 chemistry (P6/C4 for *C. malonaticus*) and three to four single-molecule real-time (SMRT) cells were used per strain with a 180-min (240 min for *C. malonaticus*) collection protocol. The subreads were *de novo* assembled using the PacBio Hierarchical Genome Assembly Process (HGAP)/Quiver software package (11), followed by minimus2 for genome circularization (12) and final polishing with Quiver. All the strains were assembled into a single contig corresponding to the chromosome. For some strains one to four circular plasmids were also obtained. The nucleotide sequences have been deposited at NCBI. The results of the sequencing and assemblies are summarized in Table 1. The genomes were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) and have been deposited at GenBank (NCBI).

TABLE 1 Summary of genome sequencing and nucleotide accession numbers

Organism	Chromosome size (bp)	No. of plasmids	Plasmid size (bp)	Accession no.
C. condimenti LMG 26250, CECT 7863^T	4,366,820	1	164,790	CP012264 to CP012265
C. muytjensii ATCC 51329^T	4,385,738	0	NA[a]	CP012268
C. sakazakii NCTC 8155	4,348,995	3	124,048/117,750/53,771	CP012253 to CP012256
C. universalis NCTC 9529^T	4,323,715	1	136,454	CP012257 to CP012258
C. malonaticus LMG 23826, DSMZ 18702^T	4,294,640	2	126,501/52,758	CP013940 to CP013942
C. dublinensis LMG 23823, DSMZ 18705^T	4,444,709	1	203,534	CP012266 to CP012267

[a] NA, not applicable.

During sequencing, epigenetic modifications of each nucleotide position were measured as kinetic variations (KVs) in nucleotide incorporation rates. Motifs were deduced from the KV data (13). Analysis were done using SMRT portal RS_Modification_and_Motif_Analysis Protocol.

Nucleotide sequence accession numbers. Sequences have been deposited in GenBank under the accession numbers listed in Table 1. Raw reads and motif summaries are deposited at SRA: *C. condimenti*^{T} SRR2154341, *C. muytjensii*^{T} SRR2154340, *C. sakazakii* SRR2154342, *C. universalis*^{T} SRR2154343, *C. malonaticus*^{T} SRR3112550, and *C. dublinensis*^{T} SRR2154345.

REFERENCES

1. **Healy B, Cooney S, O'Brien S, Iversen C, Whyte P, Nally J, Callanan JJ, Fanning S**. 2010. *Cronobacter* (*Enterobacter sakazakii*): an opportunistic foodborne pathogen. Foodborne Pathog Dis **7:**339–350. http://dx.doi.org/10.1089/fpd.2009.0379.
2. **Jaradat ZW, Al Mousa W, Elbetieha A, Al Nabulsi A, Tall BD**. 2014. *Cronobacter* spp.—opportunistic foodborne pathogens. A review of their virulence and environmental-adaptive traits. J Med Microbiol **63:**1023–1037. http://dx.doi.org/10.1099/jmm.0.073742-0.
3. **Jaradat ZW, Ababneh QO, Saadoun IM, Samara NA, Rashdan AM**. 2009. Isolation of *Cronobacter* spp. (formerly *Enterobacter sakazakii*) from infant food, herbs and environmental samples and the subsequent identification and confirmation of the isolates using biochemical, chromogenic assays, PCR and 16S rRNA sequencing. BMC Microbiol **9:**225. http://dx.doi.org/10.1186/1471-2180-9-225.
4. **Pan Z, Cui J, Lyu G, Du X, Qin L, Guo Y, Xu B, Li W, Cui Z, Zhao C**. 2014. Isolation and molecular typing of *Cronobacter* spp. in commercial powdered infant formula and follow-up formula. Foodborne Pathog Dis **11:**456–461. http://dx.doi.org/10.1089/fpd.2013.1691.
5. **Craven HM, McAuley CM, Duffy LL, Fegan N**. 2010. Distribution, prevalence and persistence of *Cronobacter* (*Enterobacter sakazakii*) in the nonprocessing and processing environments of five milk powder factories. J Appl Microbiol **109:**1044–1052. http://dx.doi.org/10.1111/j.1365-2672.2010.04733.x.
6. **Molloy C, Cagney C, O'Brien S, Iversen C, Fanning S, Duffy G**. 2009. Surveillance and characterisation by pulsed-field gel electrophoresis of *Cronobacter* spp. in farming and domestic environments, food production animals and retail foods. Int J Food Microbiol **136:**198–203. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.07.007.
7. **Iversen C, Lehner A, Mullane N, Bidlas E, Cleenwerck I, Marugg J, Fanning S, Stephan R, Joosten H**. 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. http://dx.doi.org/10.1186/1471-2148-7-64.
8. **Joseph S, Cetinkaya E, Drahovska H, Levican A, Figueras MJ, Forsythe SJ**. 2011. *Cronobacter condimenti* sp. nov., isolated from spiced meat, and *Cronobacter universalis* sp. nov., a species designation for *Cronobacter* sp. genomospecies 1, recovered from a leg infection, water and food ingredients. Int J Syst Evol Microbiol **62:**1277–1283.
9. **Baldwin A, Loughlin M, Caubilla-Barron J, Kucerova E, Manning G, Dowson C, Forsythe S**. 2009. Multilocus sequence typing of *Cronobacter sakazakii* and *Cronobacter malonaticus* reveals stable clonal structures. BMC Microbiol **9:**223. http://dx.doi.org/10.1186/1471-2180-9-223.
10. **Köser C, Ellington M, Cartwright E, Gillespie S, Brown N, Farrington M, Holden M, Dougan G, Bentley S, Parkhill J, Peacock S**. 2012. Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathog **8:**e1002824.
11. **Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J**. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods **10:**563–569. http://dx.doi.org/10.1038/nmeth.2474.
12. **Sommer DD, Delcher AL, Salzberg SL, Pop M**. 2007. Minimus: a fast, lightweight genome assembler. BMC Bioinformatics **8:**64. http://dx.doi.org/10.1186/1471-2105-8-64.
13. **Korlach J, Turner SW**. 2012. Going beyond five bases in DNA sequencing. Curr Opin Struct Biol **22:**251–261. http://dx.doi.org/10.1016/j.sbi.2012.04.002.