


Draft Genome Sequence of *Clostridium sporogenes* Strain UC9000 Isolated from Raw Milk

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***Clostridium sporogenes* is a causative agent of food spoilage and is often used as the nontoxic surrogate for *Clostridium botulinum*. Here, we described the draft genome sequence and annotation of *C. sporogenes* strain UC9000 isolated from raw milk.**

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Clostridium sporogenes represents a good microbiological model for the study of Gram-positive, spore-forming, anaerobic bacteria because it can cause food spoilage (1), it occasionally appears as a clinical pathogen (2), and it shares nearly identical metabolic properties with *Clostridium botulinum* Group I (3, 4), except for in the area of toxin production. For these reasons, it has often been used as a surrogate of *C. botulinum* in demonstrating the effectiveness of food preservation processes (4, 5) and in germination studies (6, 7). Although the analysis based on 16S rRNA sequence comparison (8, 9) grouped the two species together in a single clade, and Weingand et al. (10), on the basis of the core genome analysis, proposed that *C. sporogenes* and *C. botulinum* formed two related but separated clades, further genomic data are needed to better understand the genetic relationships between these two species and their classification in taxonomic units.

In the current work, we propose a *de novo* shotgun sequencing of *C. sporogenes* strain UC9000 isolated from raw milk and responsible of hard cheese spoilage. The genome was sequenced with a 700-fold overall genome coverage using an Illumina HiSeq 1000 platform from IGA Technology Services (Udine, Italy). The reads set were *de novo* assembled using the CLC Genomic Workbench software (version 8.0.3). This strategy resulted in 111 contigs with a calculated genome size of 4.3 Mb and a G+C content of 27.8%. A total of 4,151 genes were predicted by annotating the genome with both the NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAP) and the RAST Annotation Server (11, 12); 3,932 of the genes are coding sequences (CDS), and there are 151 pseudogenes, 1 rRNA, and 62 tRNAs.

The proteolytic nature of this strain was reflected in the genome by the presence of genes involved in amino acid metabolism. The strain also harbors genes involved in sugar metabolism, transport, and uptake, like genes participating in chitin and *N*-acetylglucosamine utilization. Genes coding for botulinism neurotoxins and accessory nontoxin-nonhemagglutinin (NTNH) were not detected. We also identified genes involved in DNA metabolism (coding for clustered regularly interspaced short palindromic repeat [CRISPR]-associated proteins and restriction-modification systems), in spore germination (*gerAA*, *gerAB*, *ge-*

rAC, *cwlJ* and *sleB* homologues), in motility and chemotaxis. Finally, two intact and three partial phage regions were identified by using the PHAge Search Tool (PHAST) (13), while transposable elements belonging to IS200/IS605 and IS66 families were retrieved from the IGS Annotation Engine and Manatee web-based tool (14).

Nucleotide sequence accession numbers. This complete genome sequence has been deposited at DDBJ/EMBL/GenBank under the accession no. [LJFK000000000](https://www.ncbi.nlm.nih.gov/nuccore/LJFK000000000). The version described in this paper is version LJFK01000000.

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REFERENCES

- McClure PJ. 2006. Spore-forming bacteria, p 579–623. In Blackburn (ed), *Food spoilage microorganisms*, vol 21. Woodhead Publishing, Sawston, United Kingdom.
- Inkster T, Cordina C, Siegmeth A. 2011. Septic arthritis following anterior cruciate ligament reconstruction secondary to *Clostridium sporogenes*; a rare clinical pathogen. *J Clin Pathol* 64:820–821. <http://dx.doi.org/10.1136/jcp.2010.084434>.
- Carter AT, Paul CJ, Mason DR, Twine SM, Alston MJ, Logan SM, Austin JW, Peck MW. 2009. Independent evolution of neurotoxin and flagellar genetic loci in proteolytic *Clostridium botulinum*. *BMC Genomics* 10:115. <http://dx.doi.org/10.1186/1471-2164-10-115>.
- Bradbury M, Greenfield P, Midgley D, Li D, Tran-Dinh N, Vriesekoop F, Brown JL. 2012. Draft genome sequence of *Clostridium sporogenes* pa 3679, the common nontoxic surrogate for proteolytic *Clostridium botulinum*. *J Bacteriol* 194:1631–1632. <http://dx.doi.org/10.1128/JB.06765-11>.
- Taylor RH, Dunn ML, Ogden LV, Jefferies LK, Eggett DL, Steele FM. 2013. Conditions associated with *Clostridium sporogenes* growth as a surrogate for *Clostridium botulinum* in nonthermally processed canned butter. *J Dairy Sci* 96:2754–2764. <http://dx.doi.org/10.3168/jds.2012-6209>.
- Brunt J, Plowman J, Gaskin DJH, Itchner M, Carter AT, Peck MW. 2014. Functional characterisation of germinant receptors in *Clostridium botulinum* and *Clostridium sporogenes* presents novel insights into spore germination systems. *PLoS Pathog*. 10:e1004382. <http://dx.doi.org/10.1371/journal.ppat.1004382>.

7. Meaney CA, Cartman ST, McClure PJ, Minton NP. 2015. Optimal spore germination in *Clostridium botulinum* ATCC 3502 requires the presence of functional copies of SleB and YpeB, but not CwlJ. *Anaerobe* 34:86–93. <http://dx.doi.org/10.1016/j.anaerobe.2015.04.015>.
8. Kalia VC, Mukherjee T, Bhushan A, Joshi J, Shankar P, Huma N. 2011. Analysis of the unexplored features of rrs (16S rDNA) of the genus *Clostridium*. *BMC Genomics* 12:18. <http://dx.doi.org/10.1186/1471-2164-12-18>.
9. Schill KM, Wang Y, Butler RR, Pombert JF, Reddy NR, Skinner GE, Larkin JW. 2015. Genetic diversity of *Clostridium sporogenes* pa 3679 isolates obtained from different sources and resolved by pulsed-field gel electrophoresis (PFGE) and high-throughput sequencing. *Appl Environ Microbiol* 82:384–393. <http://dx.doi.org/10.1128/AEM.02616-15>.
10. Weigand MR, Pena-Gonzalez A, Shirey TB, Broeker RG, Ishaq MK, Konstantinidis KT, Raphael BH. 2015. Implications of genome-based discrimination between *Clostridium botulinum* group I and *Clostridium sporogenes* strains for bacterial taxonomy. *Appl Environ Microbiol* 81:5420–5429. <http://dx.doi.org/10.1128/AEM.01159-15>.
11. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formisano 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>.
12. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Sci Rep* 5:8365. <http://dx.doi.org/10.1038/srep08365>.
13. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res* 39:W347–W352. <http://dx.doi.org/10.1093/nar/gkr485>.
14. Galens K, Orvis J, Daugherty S, Creasy HH, Angiuoli S, White O, Wortman J, Mahurkar A, Giglio MG. 2011. The IGS standard operating procedure for automated prokaryotic annotation. *Stand Genomic Sci* 4:244–251. <http://dx.doi.org/10.4056/sigs.1223234>.