





## Closed Genome Sequence of *Clostridium botulinum* Strain CFSAN064329 (62A)

Travis G. Wentz,<sup>a</sup> Kuan Yao,<sup>a</sup> Kristin M. Schill,<sup>b</sup> N. Rukma Reddy,<sup>b</sup> Guy E. Skinner,<sup>b</sup> Travis R. Morrissey,<sup>b</sup> Yun Wang,<sup>b</sup> Tim Muruvanda,<sup>a</sup> Gowri Manickam,<sup>a</sup> Christine A. Pillai,<sup>a</sup> Nagarajan Thirunavukkarasu,<sup>a</sup> Maria Hoffmann,<sup>a</sup> Thomas S. Hammack,<sup>a</sup> Eric W. Brown,<sup>a</sup> Marc W. Allard,<sup>a</sup> Shashi K. Sharma<sup>a</sup>

<sup>a</sup>Division of Microbiology, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, College Park, Maryland, USA

<sup>b</sup>Division of Food Processing Science and Technology, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, Bedford Park, Illinois, USA

**ABSTRACT** *Clostridium botulinum* is a strictly anaerobic, Gram-positive, spore-forming bacterium that produces botulinum neurotoxin, a potent and deadly proteinaceous exotoxin. *Clostridium botulinum* strain CFSAN064329 (62A) produces an A1 serotype/subtype botulinum neurotoxin and is frequently utilized in food challenge and detection studies. We report here the closed genome sequence of *Clostridium botulinum* strain CFSAN064329 (62A).

Botulinum neurotoxin (BoNT) is the causative agent of foodborne botulism, a rare and potentially deadly paralytic disease caused by oral ingestion of preformed BoNT. Organisms that produce BoNTs are usually spore-forming, strictly anaerobic, Gram-positive bacteria within the genus *Clostridium* (1). *Clostridium botulinum* is widely found in soil and marine sediments, and its endospores are highly durable and can persist on or within a variety of produce, meats, and seafood (2). Upon reintroduction of appropriate substrates and anoxic conditions, *C. botulinum* spores can germinate, enter vegetative growth, and concurrently release BoNT(s) into a food product (3, 4). Researching the conditions necessary to eliminate spores in a diverse range of food products and inhibit spore germination is important to understanding and preventing outbreaks of foodborne botulism.

Clostridium botulinum strain CFSAN064329 (62A, ATCC 7948, NCTC 7948, H 7634) is a well-characterized *C. botulinum* strain and produces an A1 serotype/subtype BoNT. The strain has been utilized in over 250 published research studies, including those exploring the resistance of spores to temperature, pH, and pressure and the regulation of toxin expression and numerous other food challenge studies, such as those investigating *C. botulinum* in extended-shelf-life refrigerated foods (5). This strain is a long-standing laboratory strain with conflicting origins; one study mentions it as having been isolated from a liver infarct of a cow with redwater disease, while another describes it as having been isolated from virgin soil samples in 1922 (6, 7).

Clostridium botulinum strain CFSAN064329 was sequenced on a Sequel platform (Pacific Biosciences, Menlo Park, CA) on a multiplexed (3-plex) run at 482× coverage and in accordance with the manufacturer's specifications. Analysis of the sequence reads was implemented using SMRT Link version 5.1. De novo assembly of the reads was performed using the PacBio HGAP.4 program, with default parameters. Overlapping regions identified at the end of the output assemblies (of chromosome and plasmids) were identified using Gepard (8). The closed assembly consists of a single chromosome of 3,920,316 bp with 28.2% GC content and a single plasmid of 10,094 bp with 26.4% GC content. The assembly was subjected to methyl motif analysis and submitted to the NCBI Prokaryotic Genome Annotation Pipeline for general annotation (9).

**Received** 1 June 2018 **Accepted** 1 June 2018 **Published** 28 June 2018

Citation Wentz TG, Yao K, Schill KM, Reddy NR, Skinner GE, Morrissey TR, Wang Y, Muruvanda T, Manickam G, Pillai CA, Thirunavukkarasu N, Hoffmann M, Hammack TS, Brown EW, Allard MW, Sharma SK. 2018. Closed genome sequence of Clostridium botulinum strain CFSAN064329 (62A). Genome Announc 6: e00528-18. https://doi.org/10.1128/genomeA

This is a work of the U.S. Government and is not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Shashi K. Sharma, Shashi.Sharma@fda.hhs.gov.

T.G.W., K.Y., and K.M.S. contributed equally to this work.

Wentz et al. genameAnnouncements™

**Accession number(s).** The complete genome sequence of *Clostridium botulinum* strain CFSAN064329 has been deposited in DDBJ/ENA/GenBank under the accession numbers CP028859 (chromosome) and CP028860 (plasmid).

## **ACKNOWLEDGMENTS**

This study was supported by funding from the Department of Homeland Security and a U.S. FDA research participation program agreement administered by the Oak Ridge Institute for Science and Education (ORISE).

## **REFERENCES**

- Johnson EA, Bradshaw M. 2001. Clostridium botulinum and its neurotoxins: a metabolic and cellular perspective. Toxicon 39:1703–1722. https://doi.org/10.1016/S0041-0101(01)00157-X.
- Dodds KL. 1992. Clostridium botulinum in the environment, p 21–52. In Hauschild AHW, Dodds KL (ed), Clostridium botulinum: ecology and control in foods, vol 54. Marcel Dekker, New York, NY.
- Treadwell PE, Jann GJ, Salle AJ. 1958. Studies on factors affecting the rapid germination of spores of *Clostridium botulinum*. J Bacteriol 76: 549–556.
- Austin JW, Dodds KL, Blanchfield B, Farber JM. 1998. Growth and toxin production by Clostridium botulinum on inoculated fresh-cut packaged vegetables. J Food Prot 61:324–328. https://doi.org/10.4315/0362-028X -61.3.324.
- Doyle M. 1991. Evaluating the potential risk from extended-shelf-life refrigerated foods by *Clostridium botulinum* inoculation studies. Food Technol 45:154–156.

- Solomon HM, Lynt RK, Jr, Kautter DA, Lilly T, Jr. 1969. Serological studies of Clostridium botulinum type E and related organisms. II. Serology of spores. J Bacteriol 98:407–414.
- 7. Hyytiä E, Björkroth J, Hielm S, Korkeala H. 1999. Characterisation of *Clostridium botulinum* groups I and II by randomly amplified polymorphic DNA analysis and repetitive element sequence-based PCR. Int J Food Microbiol 48:179–189. https://doi.org/10.1016/S0168-1605(99)00050-1.
- Krumsiek J, Arnold R, Rattei T. 2007. Gepard: a rapid and sensitive tool for creating dotplots on genome scale. Bioinformatics 23:1026–1028. https:// doi.org/10.1093/bioinformatics/btm039.
- NCBI Resource Coordinators. 2016. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 44:D7–D19. https://doi.org/10.1093/nar/gkv1290.

Volume 6 Issue 26 e00528-18 genomea.asm.org 2