AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of Historic *Clostridioides difficile* Food-Dwelling Ribotype 078 Strains in Canada Identical to That of the Historic Human Clinical Strain M120 in the United Kingdom

Anand Kumar,[a] Karen Walston Davenport,[a] Grace Vuyisich,[a] Yuliya A. Kunde,[a] Shannon Lyn Johnson,[a] Patrick Sam Guy Chain,[a] Armand Earl Ko Dichosa,[a] Alex Rodriguez-Palacios[b]

[a]B-10: Biosecurity and Public Health, Los Alamos National Laboratory, Los Alamos, New Mexico, USA

[b]Division of Gastroenterology & Liver Disease, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA

ABSTRACT *Clostridioides* (*Clostridium*) *difficile* is a spore-forming anaerobic bacterium that causes severe intestinal diseases in humans. Here, we report the complete genome sequence of the first *C. difficile* foodborne type strain (PCR ribotype 078) isolated from food animals in Canada in 2004, which has 100% similarity to the genome sequence of the historic human clinical strain M120.

The isolation of multidrug-resistant hypervirulent *Clostridioides difficile* PCR ribotype 027 and 078 strains from food animals and retail foods in Canada in 2004 (1–3) coincided with a major outbreak of severe *C. difficile* infections in humans in Canada and the United Kingdom in the same year (4, 5). Further epidemiological evidence has indicated that *C. difficile* could be a foodborne pathogen, thereby explaining a major fraction of infections acquired in hospitals and the community (6–8). Here, we applied a genome-based strategy to further test such a hypothesis and to determine the genetic features that allow highly virulent strains to move between humans and animals. We report the complete genome sequences of three historical Canadian *C. difficile* PCR ribotype 078 food strains (3) using Pacific Biosciences RS II sequencing.

In brief, pure bacterial isolates (cultured in tryptone soy agar–5% sheep blood under anaerobic conditions at 37°C; Thermo Fisher Scientific) were used for DNA extraction (QIAamp DNA blood minikit, Gram-positive bacterial protocol; Qiagen). Determinations of DNA concentration and size were performed using a Qubit fluorometer (Thermo Fisher Scientific). Genomic DNA was sheared in Covaris g-Tubes with the help of a fix-angled rotor centrifuge (Eppendorf) using the following parameters: shear for 1 min at 3,500 rpm, flip the tube, and shear for 1 min at 3,500 rpm. Sheared DNA was collected and purified using AMPure PB beads according to the PacBio protocol. Target 20-kb SMRTbell templates were prepared following an exonuclease VII reaction, DNA damage repair reaction, end repair reaction, overnight ligation, heat kill, and exonuclease III/VII digestion of misligated products. SMRTbell template size selection was performed using BluePippin (Sage Science) protocols. Sequencing primer annealing to the SMRTbell templates was followed by DNA polymerase P6 binding. SMRTbell templates bound to MagBeads were sequenced using single-molecule real-time (SMRT) version 3 cells (6Pac cells; 2 cells/isolate). The genomes were assembled and polished using the HGAP3 and Quiver software packages, respectively, within the SMRTPortal (version 2.3.0) with default settings. The genomes were additionally polished with Arrow (the resequencing pipeline run with default parameters), which is within the latest SMRT

Received 22 June 2018 **Accepted** 28 August 2018 **Published** 27 September 2018

Citation Kumar A, Davenport KW, Vuyisich G, Kunde YA, Johnson SL, Chain PSG, Dichosa AEK, Rodriguez-Palacios A. 2018. Complete genome sequences of historic *Clostridioides difficile* food-dwelling ribotype 078 strains in Canada identical to that of the historic human clinical strain M120 in the United Kingdom. Microbiol Resour Announc 7:e00853-18. https://doi.org/10.1128/MRA.00853-18.

Editor David A. Baltrus, University of Arizona

Address correspondence to Anand Kumar, akumar@lanl.gov.

TABLE 1 Genome insights and GenBank accession numbers for three *Clostridioides difficile* strains isolated from the Canadian food production system

C. difficile strain	GenBank accession no.	Genome size (bp)	No. of genes	No. of virulence genes	No. of antibiotic resistance genes	Fold coverage (×)
R1	CP026613	4,093,143	3,579	11	16	362
R2	CP026614	4,093,145	3,577	11	16	371
R3	CP026615	4,093,148	3,580	11	16	339

Analysis software bundle (SMRTLink, version 5.1.0) and checked for misassemblies, but none were identified. Annotations of the genomes were completed using Prokka within EDGE bioinformatics (9, 10).

Interestingly, whole-genome alignment and single-nucleotide polymorphism (SNP) analyses, with the help of the PhaME software (M. Shakya/P. S. G. Chain et al., unpublished data), revealed nearly 100% nucleotide sequence identity with the genome sequence of *C. difficile* strain M120 (BioProject number PRJNA42467), a PCR ribotype 078 isolate obtained from a UK patient in 2007 (11). Our whole-genome sequence analysis (Table 1) indicates that, regardless of geographic distances, these historic *C. difficile* strains concurrently had similar genomic elements to explain disease in both animals and humans.

Data availability. The complete sequences and annotations of these *C. difficile* genomes are deposited in the GenBank database with accession numbers CP026613 (*C. difficile* R1), CP026614 (*C. difficile* R2), and CP026615 (*C. difficile* R3).

ACKNOWLEDGMENTS

This project was supported by a Los Alamos National Laboratory (LANL) internal grant, the Laboratory Directed Research and Development grant 20160340ER. The research work of A.K. was supported by (i) the LANL Director's Postdoctoral Fellowship, grant 20170671PRD2, and (ii) a University of California, Davis/LANL Postdoc Entrepreneurial Fellowship. A.R.-P. received partial support from a career development award from the Crohn's and Colitis Foundation and National Institutes of Health award 2P01DK091222-06 (Germ-Free and Gut Microbiome Core, PD, Fabio Cominelli, Case Western Reserve University).

REFERENCES

1. Rodriguez-Palacios A, Ilic S, LeJeune JT. 2016. Subboiling moist heat favors the selection of enteric pathogen *Clostridium difficile* PCR ribotype 078 spores in food. Can J Infect Dis Med Microbiol 2016:1. https://doi.org/10.1155/2016/1462405.
2. Rodriguez-Palacios A, Ilic S, LeJeune JT. 2017. Food indwelling *Clostridium difficile* in naturally contaminated household meals: data for expanded risk mathematical predictions. Infect Control Hosp Epidemiol 38:509–510. https://doi.org/10.1017/ice.2016.332.
3. Rodriguez-Palacios A, Stampfli HR, Duffield T, Peregrine AS, Trotz-Williams LA, Arroyo LG, Brazier JS, Weese JS. 2006. *Clostridium difficile* PCR ribotypes in calves, Canada. Emerg Infect Dis 12:1730–1736. https://doi.org/10.3201/eid1211.051581.
4. McDonald LC, Killgore GE, Thompson A, Owens RC, Kazakova SV, Sambol SP, Johnson S, Gerding DN. 2005. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N Engl J Med 353:2433–2441. https://doi.org/10.1056/NEJMoa051590.
5. Warny M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, Frost E, McDonald LC. 2005. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. Lancet 366:1079–1084. https://doi.org/10.1016/S0140-6736(05)67420-X.
6. Eyre DW, Cule ML, Wilson DJ, Griffiths D, Vaughan A, O'Connor L, Ip CLC, Golubchik T, Batty EM, Finney JM, Wyllie DH, Didelot X, Piazza P, Bowden R, Dingle KE, Harding RM, Crook DW, Wilcox MH, Peto TEA, Walker AS. 2013. Diverse sources of *C. difficile* infection identified on whole-genome sequencing. N Engl J Med 369:1195–1205. https://doi.org/10.1056/NEJMoa1216064.
7. Rodriguez-Palacios A, Borgmann S, Kline TR, Lejeune JT. 2013. *Clostridium difficile* in foods and animals: history and measures to reduce exposure. Anim Health Res Rev 14:11–29. https://doi.org/10.1017/S1466252312000229.
8. Søes LM, Holt HM, Böttiger B, Nielsen HV, Andreasen V, Kemp M, Olsen KEP, Ethelberg S, Mølbak K. 2014. Risk factors for *Clostridium difficile* infection in the community: a case-control study in patients in general practice, Denmark, 2009–2011. Epidemiol Infect 142:1437–1449. https://doi.org/10.1017/S0950268813002380.
9. Li P-E, Lo C-C, Anderson JJ, Davenport KW, Bishop-Lilly KA, Xu Y, Ahmed S, Feng SH, Mokashi VP, Chain PSG. 2017. Enabling the democratization of the genomics revolution with a fully integrated Web-based bioinformatics platform. Nucleic Acids Res 45:67–80. https://doi.org/10.1093/nar/gkw1027.
10. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
11. He M, Sebaihia M, Lawley TD, Stabler RA, Dawson LF, Martin MJ, Holt KE, Seth-Smith HMB, Quail MA, Rance R, Brooks K, Churcher C, Harris D, Bentley SD, Burrows C, Clark L, Corton C, Murray V, Rose G, Thurston S, van Tonder A, Walker D, Wren BW, Dougan G, Parkhill J. 2010. Evolutionary dynamics of *Clostridium difficile* over short and long time scales. Proc Natl Acad Sci U S A 107:7527–7532. https://doi.org/10.1073/pnas.0914322107.