AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Draft Genome Sequence of *Clostridium bowmanii* DSM 14206^T, Isolated from an Antarctic Microbial Mat

Faith P. Palevich,[a] Nikola Palevich,[b] Paul H. Maclean,[b] Eric Altermann,[a,c] John Mills,[a] Gale Brightwell[a,d]

[a]AgResearch Ltd., Hopkirk Research Institute, Palmerston North, New Zealand
[b]AgResearch Ltd., Grasslands Research Centre, Palmerston North, New Zealand
[c]Riddet Institute, Massey University, Palmerston North, New Zealand
[d]New Zealand Food Safety Science and Research Centre, Massey University, Palmerston North, New Zealand

ABSTRACT *Clostridium bowmanii* type strain DSM 14206 (ATCC BAA-581) was isolated from a microbial mat sample retrieved from Lake Fryxell, Antarctica. This report describes the generation and annotation of the 4.9-Mb draft genome sequence of *C. bowmanii* DSM 14206^T.

Clostridium bowmanii DSM 14206^T (ATCC BAA-581^T) is a Gram-positive, spore-forming, and slow-growing psychophilic anaerobe with an optimum temperature range of 12°C to 16°C (1) that was originally isolated from a mat sample from the shallows surrounding a moat on the Antarctic Lake Fryxell (2). *C. bowmanii* has been identified as a spoilage microorganism in chilled meat products and vacuum-packed meat without the production of gas. While initial phylogenetic analysis based on 16S rRNA gene sequence data placed DSM 14206^T closest to the clostridial cluster containing *Clostridium estertheticum* type strain DSM 8809 (ATCC 51377) (2), subsequent 16S rRNA gene and phylogenomic analyses placed DSM 14206^T closest to *Clostridium tagluense* DSM 17763^T (3) and *Clostridium* sp. strain FP3, which was cultivated from a spoiled chilled vacuum pack of lamb meat (4–6).

Strain DSM 14206^T was acquired from the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures) and cultured anaerobically at 12°C in prereduced peptone-yeast extract-glucose-starch (PYGS) broth (7). Genomic DNA was extracted using a modified phenol-chloroform procedure (8) and was mechanically sheared using a nebulizer instrument (Invitrogen) to select fragments of approximately 550 bp. A DNA library was prepared using the Illumina TruSeq Nano method and sequenced on the Illumina MiSeq platform with the 2 × 250-bp paired-end (PE) reagent kit v2, producing a total of 4,394,200 PE raw reads. The A5-miseq pipeline v20169825 with standard parameters was used to check the quality of the raw reads and subsequent read trimming and assembly (9). In addition, the *de novo* assembly of the MiSeq reads was merged with the publicly available DSM 14206^T assembly (GenBank assembly accession number ASM1886131v1) using Quickmerge v0.3 (10). The trimmed Illumina reads were mapped back to the refined assembly using BWA v0.7.17-r1188 (11), and contigs to which no unique reads mapped were removed.

The reported and improved assembly consists of 92 contigs, with 202× coverage and an N_{50} value of 134,761 bp; the largest scaffold is 280,085 bp in length. The draft genome sequence is composed of 4,882,709 bp, with a G+C content of 31.2%. A total of 4,546 putative protein-coding genes (PCGs), along with 88 tRNA and 16 rRNA elements, were predicted using Prokka v1.14.5 (12) and GAMOLA2 (13). All bioinformatic analyses were performed using default settings and parameters.

Overall, we produced an improved draft genome sequence of DSM 14206^T, with increased sequence coverage and quality of genomic information but with a significant

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

Address correspondence to Faith P. Palevich, nik.palevich@agresearch.co.nz.

The authors declare no conflict of interest.

Received 19 October 2021
Accepted 4 December 2021
Published 6 January 2022

reduction in the number of contigs, by combining Illumina sequence technologies. Additional annotation via carbohydrate-active enzyme profiling using dbCAN2 (14) revealed that the DSM 14206^T genome encodes 54 glycoside hydrolases, 53 glycosyl transferases, 1 polysaccharide lyase, 20 carbohydrate esterases, and 7 carbohydrate-binding protein module families. Clustered regularly interspaced short palindromic repeats (CRISPRs) were identified, with a large 7.7-kb CRISPR1-Cas with flanking *cas* genes on scaffold 35. The draft DSM 14206^T genome sequence reported here is a valuable resource for future studies investigating psychrophilic *Clostridium* species and their spoilage properties.

Data availability. The genome sequence and associated data for *Clostridium bowmanii* type strain DSM 14206 (ATCC BAA-581) were deposited under GenBank accession number JAIRAZ000000000, BioProject accession number PRJNA574489, and Sequence Read Archive (SRA) accession number SRR15734597.

ACKNOWLEDGMENTS

We thank Xiaoxiao Lin from the Massey Genome Service, Massey University, New Zealand, for assistance with the DNA sequencing.

This work was completed under the Food integrity: doing the right things in a way that builds trust project and was funded by the New Zealand Ministry of Business, Innovation, and Employment Strategic Science Investment Fund (contract C10X1702).

REFERENCES

1. Spring S, Merkhoffer B, Weiss N, Kroppenstedt RM, Hippe H, Stackebrandt E. 2003. Characterization of novel psychrophilic clostridia from an Antarctic microbial mat: description of *Clostridium frigoris* sp. nov., *Clostridium lacusfryxellense* sp. nov., *Clostridium bowmanii* sp. nov. and *Clostridium psychrophilum* sp. nov. and reclassification of *Clostridium laramiense* as *Clostridium estertheticum* subsp. *laramiense* subsp. nov. Int J Syst Evol Microbiol 53:1019–1029. https://doi.org/10.1099/ijs.0.02554-0.
2. Brambilla E, Hippe H, Hagelstein A, Tindall BJ, Stackebrandt E. 2001. 16S rDNA diversity of cultured and uncultured prokaryotes of a mat sample from Lake Fryxell, McMurdo Dry Valleys, Antarctica. Extremophiles 5:23–33. https://doi.org/10.1007/s007920000169.
3. Dorn-In S, Schwaiger K, Springer C, Barta L, Ulrich S, Gareis M. 2018. Development of a multiplex qPCR for the species identification of *Clostridium estertheticum*, *C. frigoriphilum*, *C. bowmanii* and *C. tagluense*-like from blown pack spoilage (BPS) meats and from wild boars. Int J Food Microbiol 286:162–169. https://doi.org/10.1016/j.ijfoodmicro.2018.08.020.
4. Palevich N, Palevich FP, Maclean PH, Jauregui R, Altermann E, Mills J, Brightwell G. 2020. Draft genome sequence of *Clostridium estertheticum*-like strain FP3, isolated from spoiled uncooked lamb. Microbiol Resour Announc 9:e00434-20. https://doi.org/10.1128/MRA.00434-20.
5. Wambui J, Cernela N, Stevens MJA, Stephan R. 2021. Whole genome sequence-based identification of *Clostridium estertheticum* complex strains supports the need for taxonomic reclassification within the species *Clostridium estertheticum*. Front Microbiol 12:727022. https://doi.org/10.3389/fmicb.2021.727022.
6. Palevich N, Palevich FP, Maclean PH, Altermann E, Gardner A, Burgess S, Mills J, Brightwell G. 2021. Comparative genomics of *Clostridium* species associated with vacuum-packed meat spoilage. Food Microbiol 95:103687. https://doi.org/10.1016/j.fm.2020.103687.
7. Lund BM, Graham AF, George SM, Brown D. 1990. The combined effect of incubation temperature, pH and sorbic acid on the probability of growth of non-proteolytic, type B *Clostridium botulinum*. J Appl Bacteriol 69:481–492. https://doi.org/10.1111/j.1365-2672.1990.tb01539.x.
8. Bouillaut L, McBride SM, Sorg JA. 2011. Genetic manipulation of *Clostridium difficile*. Curr Protoc Microbiol Chapter 9:Unit 9A.2. https://doi.org/10.1002/9780471729259.mc09a02s20.
9. Coil D, Jospin G, Darling AE. 2015. A5-miseq: an updated pipeline to assemble microbial genomes from Illumina MiSeq data. Bioinformatics 31:587–589. https://doi.org/10.1093/bioinformatics/btu661.
10. Chakraborty M, Baldwin-Brown JG, Long AD, Emerson J. 2016. Contiguous and accurate de novo assembly of metazoan genomes with modest long read coverage. Nucleic Acids Res 44:e147.
11. Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303.3997. https://arxiv.org/abs/1303.3997.
12. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
13. Altermann E, Lu J, McCulloch A. 2017. GAMOLA2, a comprehensive software package for the annotation and curation of draft and complete microbial genomes. Front Microbiol 8:346. https://doi.org/10.3389/fmicb.2017.00346.
14. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y. 2018. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res 46:W95–W101. https://doi.org/10.1093/nar/gky418.