



# Draft Genome Sequence of *Clostridium estertheticum*-Like Strain FP3, Isolated from Spoiled Uncooked Lamb

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**ABSTRACT** *Clostridium estertheticum*-like strain FP3 was isolated from vacuum-packaged refrigerated spoiled lamb. This bacterium is psychrotrophic, Gram positive, spore-forming, and a strict anaerobe. Here, we report the generation and annotation of the 5.6-Mb draft genome sequence of *C. estertheticum*-like strain FP3.

Several bacterial species of the genus *Clostridium* have been recognized in occurrences of blown-pack spoilage (BPS), predominantly *C. estertheticum* (1). The potential of a BPS episode is one of the primary reasons that abattoirs cannot adopt more cost-effective techniques that would provide substantial improvements in quality meat production. *Clostridium estertheticum*-like strain FP3 is a Gram-positive, spore-forming, and slow-growing psychrotrophic anaerobe, originally isolated from vacuum-packaged refrigerated spoiled lamb at AgResearch Ltd., Palmerston North, New Zealand. Existing molecular-based detection strategies grouped FP3 into the non-toxicogenic *C. estertheticum*-like cluster with >89% similarity based on recent amplified rDNA (ribosomal DNA) restriction analysis (ARDRA) of psychrotolerant *Clostridium* isolates (2), in addition to a positive test with the real-time *C. estertheticum*-like-specific PCR assay (3).

Strain FP3 was isolated in 2017 from the meat drip of a fully distended pack of vacuum-packaged lamb in which the meat was discolored (gray-brown). FP3 was cultured anaerobically from the meat drip at 10°C in 10-fold suspensions of prereduced peptone-yeast extract-glucose-starch (PYGS) broth (3). Genomic DNA was extracted using a modified phenol-chloroform procedure as previously described (4–6). Genomic DNA was prepared for whole-genome sequencing using an Illumina TruSeq Nano library preparation kit (Illumina, Inc., San Diego, CA) according to the manufacturer's instructions, and sequencing was performed using a MiSeq instrument (Illumina, Inc.) with 250-bp paired-end sequencing. In total, 3,189,796 raw reads were generated. Trimming and *de novo* assembly were performed using the A5-miseq pipeline v20169825 (7), with QAST v5.0.0 (8) used to assess the assembly scaffold quality, such as the number of contigs, G+C content,  $N_{50}$  value, and total size. The result was a 5,555,543-bp draft genome assembly with 85 contigs, an average coverage of 139×, an  $N_{50}$  value of 245,874 bp, with the largest scaffold being 919,536 bp, and a G+C content of 31.5%.

The FP3 draft genome was initially annotated using the GAMOLA2 (9) and OmicsBox v1.1.164 (10) software packages, with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) software package (11) used to generate the final annotation. Default parameters were used for all software unless otherwise specified. Annotated features include 5,023 putative protein-coding genes (PCGs) with 101 tRNAs, 60 rRNAs, and 150 noncoding RNA (ncRNA) elements. Functional annotation of the carbohydrate-active enzymes (12) implies that different members of the *Clostridium estertheticum* species vary in their ability to utilize simple carbohydrates for growth, especially for complex

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polysaccharides. dbCAN2 (13) predicted 48 glycoside hydrolases (GHs), 30 glycosyl transferases (GTs), 5 carbohydrate esterases (CEs), and 9 carbohydrate-binding protein module (CBM) families but no polysaccharide lyases (PLs). Interestingly, FP3 encodes half as many CBMs and approximately 15 fewer GHs than the closely related *C. estertheticum* type strains (5, 14). In conclusion, this draft genome sequence of *C. estertheticum*-like strain FP3 will facilitate future functional genomic studies investigating the bacterial genetic mechanisms associated with BPS.

**Data availability.** The project data for *Clostridium estertheticum*-like strain FP3 have been submitted under GenBank accession number [JAAMNH0000000000](https://www.ncbi.nlm.nih.gov/GenBank/FASTA/JAAMNH0000000000) and BioProject accession number [PRJNA574489](https://www.ncbi.nlm.nih.gov/BioProject/PRJNA574489) and the raw sequences under Sequence Read Archive (SRA) accession number [SRR11113221](https://www.ncbi.nlm.nih.gov/SRA/SRR11113221).

## REFERENCES

- Mills J, Donnison A, Brightwell G. 2014. Factors affecting microbial spoilage and shelf-life of chilled vacuum-packed lamb transported to distant markets: a review. *Meat Sci* 98:71–80. <https://doi.org/10.1016/j.meatsci.2014.05.002>.
- Brightwell G, Horváth KM. 2018. Molecular discrimination of New Zealand sourced meat spoilage associated psychrotolerant *Clostridium* species by ARDRA and its comparison with 16S RNA gene sequencing. *Meat Sci* 138:23–27. <https://doi.org/10.1016/j.meatsci.2017.12.007>.
- Brightwell G, Clemens R. 2012. Development and validation of a real-time PCR assay specific for *Clostridium estertheticum* and *C. estertheticum*-like psychrotolerant bacteria. *Meat Sci* 92:697–703. <https://doi.org/10.1016/j.meatsci.2012.06.025>.
- Bouillaut L, McBride SM, Sorg JA. 2011. Genetic manipulation of *Clostridium difficile*. *Curr Protoc Microbiol* Chapter 9:Unit 9A.2.
- Palevich N, Palevich FP, Maclean PH, Jauregui R, Altermann E, Mills J, Brightwell G. 2019. Draft genome sequence of *Clostridium estertheticum* subsp. *laramiense* DSM 14864<sup>T</sup>, isolated from spoiled uncooked beef. *Microbiol Resour Announc* 8:e01275-19. <https://doi.org/10.1128/MRA.01275-19>.
- Palevich N, Palevich FP, Maclean PH, Jauregui R, Altermann E, Mills J, Brightwell G. 2020. Draft genome sequence of psychrotolerant *Clostridium* sp. strain M14, isolated from spoiled uncooked venison. *Microbiol Resour Announc* 9:e00314-20. <https://doi.org/10.1128/MRA.00314-20>.
- 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>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
- 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>.
- Conesa A, Götz S, García-Gómez JM, Terol J, Talón M, Robles M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21:3674–3676. <https://doi.org/10.1093/bioinformatics/bti610>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
- Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res* 37:D233–D238. <https://doi.org/10.1093/nar/gkn663>.
- 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>.
- Yu Z, Gunn L, Brennan E, Reid R, Wall PG, Gaora PÓ, Hurley D, Bolton D, Fanning S. 2016. Complete genome sequence of *Clostridium estertheticum* DSM 8809, a microbe identified in spoiled vacuum packed beef. *Front Microbiol* 7:1764. <https://doi.org/10.3389/fmicb.2016.01764>.