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Year: 2020

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DOI: https://doi.org/10.1128/mra.00806-20

Posted at the Zurich Open Repository and Archive, University of Zurich ZORA URL: https://doi.org/10.5167/uzh-200141 Journal Article Published Version



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#### Originally published at:

Wambui, Joseph; Cernela, Nicole; Stevens, Marc J A; Stephan, Roger (2020). Draft genome sequence of Clostridium estertheticum CEST001, belonging to a novel subspecies of C. estertheticum, isolated from chilled vacuum-packed lamb meat imported to Switzerland. Microbiology Resource Announcements, 9(33):e00806-20.

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## Draft Genome Sequence of Clostridium estertheticum CEST001, Belonging to a Novel Subspecies of C. estertheticum, Isolated from Chilled Vacuum-Packed Lamb Meat Imported to **Switzerland**

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ABSTRACT We present the draft genome sequence of Clostridium estertheticum strain CEST001. The genome is 4.8 Mbp long with a GC content of 30.6%. The digital DNA-DNA hybridization values against four C. estertheticum strains indicate that C. estertheticum CEST001 belongs to a novel subspecies of C. estertheticum.

lostridium estertheticum is a spore-forming anaerobic psychrophilic bacterium causing blown-pack spoilage (BPS) in chilled vacuum-packed meat (1). Here, we determined the draft genome sequence of  $\beta$ -hemolytic C. estertheticum CEST001, isolated from chilled vacuum-packed lamb meat imported from New Zealand to Switzerland (2).

Isolation of the strain was carried out anaerobically at 4°C in a multistep process involving ethanol and lysozyme treatment followed by enrichment in peptone yeast glucose starch medium for 3 weeks and plating and incubation for 3 weeks on Columbia agar supplemented with 5% defibrinated sheep blood (CBA). The strain was further subcultured on CBA for 2 weeks at 4°C for genomic DNA extraction.

Genomic DNA was isolated using the DNA blood and tissue kit (Qiagen, Hombrechtikon, Switzerland). Sequencing libraries were prepared using Nextera DNA Flex chemistry (Illumina, San Diego, CA, USA), and the resulting transposome-based libraries were sequenced on a MiniSeq sequencer (Illumina). The sequencing output was 277 Mbp paired-end reads of 150 to 300 bp. Reads were checked for quality using the software package FastQC 0.11.7 (3) and then assembled using the SPAdes 3.0-based software (4) Shovill 1.0.4 (https://github.com/tseemann/shovill). The assembly was filtered, retaining contigs of >500 bp. The genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (5) and the RAST pipeline (6). Default parameters were used for all software and Web servers.

The genome was assembled into 65 contigs and comprises 4.8 Mbp with a GC content of 30.6%, 4,678 genes, and 4,563 coding sequences. The genome coverage, contig  $N_{50}$  value, and contig  $L_{50}$  value are 50.0×, 222,559 bp, and 8, respectively. The 16S rRNA gene sequence was determined in silico (7) and used in the strain identification using the 16S-based identification tool (7). The strain was identified as C. estertheticum. The results were validated by whole-genome sequencing (WGS) using digital DNA-DNA hybridization (dDDH) (8) and average nucleotide identity (ANI) (9) against the WGS of four C. estertheticum strains (10-12). ANI and dDDH values for the identification of species ranged from 96.6 to 96.8% and 81.3 to 82.9%, respectively, which are above the 95% and 70% thresholds, respectively (8, 13, 14). However, the dDDH value for the identification of subspecies ranged from 32.1 to 34.1%, which is below the 79% threshold (8). This indicates that C. estertheticum CEST001 belongs to a novel subspecies of C. estertheticum.

tRNAscan-SE (15) identified 89 tRNAs. RAST (6) identified 104 RNAs and 34 features

Citation Wambui J, Cernela N, Stevens MJA, Stephan R. 2020. Draft genome sequence of Clostridium estertheticum CEST001, belonging to a novel subspecies of C. estertheticum, isolated from chilled vacuum-packed lamb meat imported to Switzerland. Microbiol Resour Announc 9:e00806-20. https://doi.org/ 10.1128/MRA.00806-20.

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

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Received 13 July 2020 Accepted 24 July 2020 Published 13 August 2020 for antibiotic and metal tolerance. VFDB 2019 (16) predicted 32 potential virulence factors, but the strain was predicted to be a nonhuman pathogen by PathogenFinder (17).

The present genome sequence adds to the short list of publicly available *C. estertheticum* genome sequences.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession no. JABEYB00000000. The version described in this paper is version JABEYB010000000. The raw sequencing reads have been deposited in the SRA under the accession no. SRR11775223.

#### **REFERENCES**

- 1. Wambui J, Stephan R. 2019. Relevant aspects of *Clostridium estertheticum* as a specific spoilage organism of vacuum-packed meat. Microorganisms 7:142. https://doi.org/10.3390/microorganisms7050142.
- Wambui J, Püntener S, Corti S, Cernela N, Stephan R. 2020. Detection of psychrophilic *Clostridium* spp. causing "blown pack" spoilage in meat juice samples from chilled vacuum-packed beef and lamb meat imported from different countries to Switzerland. J Food Prot 83:56–59. https://doi.org/10.4315/0362-028X.JFP-19-321.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/ fastqc/.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity GM, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an online repository of standard operating procedures (SOPS) for (meta)genomic annotation. OMICS 12:137–141. https://doi.org/10.1089/omi.2008.0017.
- 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. https:// doi.org/10.1038/srep08365.
- Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67: 1613–1617. https://doi.org/10.1099/ijsem.0.001755.
- Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequencebased species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60. https://doi.org/10.1186/ 1471-2105-14-60.
- 9. Yoon SH, Min Ha S, Lim J, Kwon S, Chun J. 2017. A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van

- Leeuwenhoek 110:1281–1286. https://doi.org/10.1007/s10482-017 -0844-4.
- 10. Yu Z, Gunn L, Brennan E, Reid R, Wall PG, Gaora P, 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 7:1–10. https://doi.org/10.3389/fmicb.2016.01764.
- 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.
- Zhang P, Ward P, McMullen LM, Yang X. 2020. A case of "blown pack" spoilage of vacuum-packaged pork likely associated with Clostridium estertheticum in Canada. Lett Appl Microbiol 70:13–20. https://doi.org/ 10.1111/lam.13236.
- Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME. 2018. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0 .002516.
- 14. Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, Fiebig A, Rohde C, Rohde M, Fartmann B, Goodwin LA, Chertkov O, Reddy TBK, Pati A, Ivanova NN, Markowitz V, Kyrpides NC, Woyke T, Göker M, Klenk HP. 2014. Complete genome sequence of DSM 30083<sup>T</sup>, the type strain (U5/41<sup>T</sup>) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. Stand Genomic Sci 9:2. https://doi.org/10.1186/1944-3277-9-2.
- Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. Nucleic Acids Res 44: W54–W57. https://doi.org/10.1093/nar/gkw413.
- Liu B, Zheng D, Jin Q, Chen L, Yang J. 2019. VFDB 2019: a comparative pathogenomic platform with an interactive Web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/gky1080.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 67:2640–2644. https://doi.org/10.1093/jac/dks261.