





Draft Genome Sequence of Campylobacter fetus subsp. fetus CITCf01, Isolated from a Patient with Subacute Bacterial **Endocarditis**

Eamonn P. Culligan, a James O'Connor, Caoimhe Lynch, Deirdre O'Brien, Carl Vaughan, Declan Bolton, Aidan Coffey, a Roy D. Sleator, a Brigid Luceya

ABSTRACT Campylobacter fetus is a Gram-negative, zoonotic pathogen and a member of the class Epsilonproteobacteria. We report the draft genome sequence of C. fetus subsp. fetus CITCf01, isolated from a patient with subacute bacterial endocarditis. CITCf01 grew under aerobic, microaerobic, and anaerobic conditions, and at 42°C, an unusual combination of growth conditions.

ampylobacter fetus is a pathogen of animals and humans. Three subspecies have been characterized to date, namely C. fetus subsp. fetus, C. fetus subsp. venerealis, and C. fetus subsp. testudinum. C. fetus subsp. testudinum is primarily associated with reptiles (1, 2), while C. fetus subsp. fetus and C. fetus subsp. venerealis are associated with human and animal infections (3, 4). Invasive C. fetus infections in humans have included meningitis, prosthetic hip joint infection, bacteremia, pericarditis, endocarditis, and infectious aortitis, among others (5-11).

C. fetus subsp. fetus CITCf01 (hereafter referred to as CITCf01) was isolated from three peripheral blood culture sets (FAN BacT/Alert, bioMérieux) taken 1 day apart, after informed patient consent was obtained. The Virtuo BacT/Alert (bioMérieux) blood culture microbial detection system flagged both aerobic and anaerobic bottles positive for each set. The time-to-positivity (TTP) ranged from 40 hours 43 min to 62 hours 24 min (mean TTP 52 hours 8 min). Gram stain of cultures from each bottle were reported as curved, Gram-negative bacilli, typical of Campylobacter species.

Subcultures of the positive culture bottles grew microaerobically (at 35°C, 37°C, and 42°C) on selective charcoal-cefoperazone deoxycholate agar (CCDA) (Oxoid). CITCf01 grew in a 5% CO₂-enriched aerobic environment, microaerobically and anaerobically, at 35°C on Columbia blood agar (CBA) (Oxoid) and on Mueller-Hinton agar. Furthermore, CITCf01 was cultured microaerobically from frozen stocks on blood-free Campylobacter selectivity agar (Sigma-Aldrich, USA). Subculturing on CBA confirmed weak growth under aerobic conditions at 37°C. The initial clinical identification of CITCf01 was by Vitek 2 biochemical analytical profile using the Neisseria-Haemophilus (NH) identification card and by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (Bruker Daltronics).

CITCf01 genomic DNA was isolated using the GenElute genomic DNA kit (Sigma-Aldrich, USA), in accordance with the manufacturer's instructions. Library preparation, whole-genome sequencing (WGS), and subsequent assembly were performed by MicrobesNG (University of Birmingham, UK) using the following methods.

DNA was quantified in triplicate with the Quant-iT double-stranded DNA (dsDNA) high sensitivity assay kit (Thermo Fisher, USA). Genomic DNA libraries were prepared Citation Culligan EP, O'Connor J, Lynch C, O'Brien D, Vaughan C, Bolton D, Coffey A, Sleator RD, Lucey B. 2019. Draft genome sequence of Campylobacter fetus subsp. fetus CITCf01, isolated from a patient with subacute bacterial endocarditis. Microbiol Resour Announc 8:e01556-18. https://doi.org/10.1128/ MRA.01556-18.

Editor Irene L. G. Newton, Indiana University, Bloomington

Copyright © 2019 Culligan et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Eamonn P. Culligan, eamonn.culligan@cit.ie.

E.P.C. and J.O. contributed equally to this work.

Received 27 November 2018 Accepted 13 February 2019 Published 14 March 2019

^aDepartment of Biological Sciences, Cork Institute of Technology, Bishopstown, Cork, Ireland

Department of Clinical Microbiology, Mercy University Hospital, Grenville Place, Cork, Ireland

^cDepartment of Cardiology, Mercy University Hospital, Grenville Place, Cork, Ireland

dTeagasc Food Research Centre, Ashtown, Dublin, Ireland



using the Nextera-XT protocol (Illumina, San Diego, USA) with the following exceptions: 2 ng of input DNA was used instead of 1 ng, and PCR elongation time was increased from 30 seconds to 1 min. DNA quantification and library preparation were performed on a Hamilton Microlab STAR system. Pooled libraries were quantified using the Kapa Biosystems library quantification kit on a Roche light cycler 96 quantitative PCR (qPCR) machine. Libraries were sequenced on the Illumina HiSeq using a 250-bp paired-end protocol. Reads were adapter trimmed using Trimmomatic 0.30 with a sliding window quality cutoff of Q15 (12). *De novo* assembly was performed on samples using SPAdes version 3.7.0 (13). Quality of assembly and predicted genome size were assessed using QUAST (14). WGS generated 75,582 reads, of which 72,785 passed quality filtering. Genome assembly generated a total of 55 contigs with a mean coverage of 18.96× and 42 contigs \geq 200 bp. Following removal of low-coverage (<1.0×) contigs, the predicted genome size is approximately 1.75 Mb, with a G+C content of 33.22%. The genome was annotated by using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (15) and is predicted to contain 1,778 genes (1,707 coding genes).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number RBHV00000000. The version described in this paper is the first version, RBHV01000000. Raw reads were submitted to SRA and are available via accession number PRJNA493797.

ACKNOWLEDGMENTS

E.P.C. is funded by a Science Foundation Ireland Postdoctoral Industry Fellowship (16/IFA/4354). C.L. is funded by the Department of Agriculture Food and the Marine (DAFM), Food Institutional Research Measure (FIRM) (15/F/641).

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

- Fitzgerald C, Tu ZC, Patrick M, Stiles T, Lawson AJ, Santovenia M, Gilbert MJ, van Bergen M, Joyce K, Pruckler J, Stroika S, Duim B, Miller WG, Loparev V, Sinnige JC, Fields PI, Tauxe RV, Blaser MJ, Wagenaar JA. 2014. Campylobacter fetus subsp. testudinum subsp. nov., isolated from humans and reptiles. Int J Syst Evol Microbiol 64:2944–2948. https://doi.org/10.1099/ijs.0.057778-0.
- Gilbert MJ, Miller WG, Yee E, Zomer AL, van der Graaf-van Bloois L, Fitzgerald C, Forbes KJ, Méric G, Sheppard SK, Wagenaar JA, Duim B. 2016. Comparative genomics of *Campylobacter fetus* from reptiles and mammals reveals divergent evolution in host-associated lineages. Genome Biol Evol 8:2006–2019. https://doi.org/10.1093/gbe/evw146.
- Sahin O, Yaeger M, Wu Z, Zhang Q. 2017. Campylobacter-associated diseases in animals. Annu Rev Anim Biosci 5:21–42. https://doi.org/10.1146/annurev-animal-022516-022826.
- Wagenaar JA, van Bergen MAP, Blaser MJ, Tauxe RV, Newell DG, van Putten JPM. 2014. Campylobacter fetus infections in humans: exposure and disease. Clin Infect Dis 58:1579–1586. https://doi.org/10.1093/cid/ciu085.
- Chen Y, Liao K, Huang H, Guo P, Wu Z. 2017. Bacteremia caused by Campylobacter fetus in a patient with hepatitis B virus infection complicated with alcoholic liver cirrhosis. Clin Lab 63:1523–1525. https://doi .org/10.7754/Clin.Lab.2017.170415.
- Ishihara A, Hashimoto E, Ishioka H, Kobayashi H, Gomi H. 2018. Campylobacter fetus meningitis associated with eating habits of raw meat and raw liver in a healthy patient: a case report and literature review. IDCases 11:97–100. https://doi.org/10.1016/j.idcr.2018.02.002.
- Moon J, Kim N, Lee HS, Shin H-R, Lee S-T, Jung K-H, Park K-I, Lee SK, Chu K. 2017. Campylobacter fetus meningitis confirmed by a 16S rRNA gene analysis using the MinION nanopore sequencer, South Korea, 2016. Emerg Microbes Infect 6:e94. https://doi.org/10.1038/emi.2017.81.

- Zamora-López MJ, Álvarez-García P, García-Campello M. 2018. Prosthetic hip joint infection caused by *Campylobacter fetus*: a case report and literature review. Rev Esp Quimioter 31:53–57.
- Agrawal A, Sikachi RR. 2016. Infective abdominal aortitis due to Campylobacter fetus bacteremia: a case report and review of literature. Intractable Rare Dis Res 5:290–293. https://doi.org/10.5582/irdr.2016.01059.
- Kiyasu Y, Akiyama D, Kurihara Y, Koganemaru H, Hitomi S. 2017. Pericarditis caused by Campylobacter fetus subspecies fetus associated with ingestion of raw beef liver. J Infect Chemother 23:833–836. https://doi.org/10.1016/j.ijac.2017.07.010.
- Sękowska A, Fabiszak T, Mikucka A, Andrzejewska M, Kruszyńska E, Gospodarek E, Klawe J. 2016. A case of defibrillator-associated infective endocarditis due to *Campylobacter fetus*. Folia Microbiol (Praha) 61:529–532. https://doi.org/10.1007/s12223-016-0466-7.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10 .1093/bioinformatics/btu170.
- 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.
- 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.
- 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.

Volume 8 Issue 11 e01556-18 mra.asm.org **2**