



# Genome Sequence of *Eubacterium callanderi* AMC0717, Isolated from the Colonic Mucosa of an 11-Year-Old Organ Donor

Alan J. Marsh,<sup>a,b</sup> Kshipra Chandrashekar,<sup>a,b</sup> Sandy Ng,<sup>a,b</sup> Jeff Roach,<sup>b,c</sup> Scott T. Magness,<sup>a,d,e</sup>  M. Andrea Azcarate-Peril<sup>a,b</sup>

<sup>a</sup>Department of Medicine, Division of Gastroenterology and Hepatology, School of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA

<sup>b</sup>UNC Microbiome Core, Center for Gastrointestinal Biology and Disease, School of Medicine, University of North Carolina, Chapel Hill, North Carolina, USA

<sup>c</sup>UNC Information Technology Services and Research Computing, University of North Carolina, Chapel Hill, North Carolina, USA

<sup>d</sup>Joint Department of Biomedical Engineering, UNC/NC State University, Chapel Hill, North Carolina, USA

<sup>e</sup>Department of Cell Biology and Physiology, University of North Carolina, Chapel Hill, North Carolina, USA

**ABSTRACT** *Eubacterium callanderi* AMC0717 was isolated from the mucosa of the transverse colon of an 11-year-old organ donor. This strain contains genes putatively encoding short-chain fatty acids (SCFAs), exopolysaccharide (EPS), and several B vitamins.

*Eubacterium callanderi* is an anaerobic, non-spore-forming member of the phylum *Firmicutes*, belonging to the true *Eubacterium* cluster within the *Clostridium* XV complex (1). *E. callanderi* has thus far only been isolated from ruminal content and pig feces (2). While *E. callanderi* has been reported as the causative agent in a single case of bacteremia (3), strains are currently listed under biosafety level 1 in the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ).

Dilutions from the mucosa of the transverse colon of an 11-year-old organ donor were plated onto thioglycolate agar and grown in an anaerobic chamber, after which a pure colony of *Eubacterium callanderi* AMC0717 was isolated. Notably, unlike some of the strains listed in culture collections, AMC0717 can be cultivated without rumen fluid in pre-reduced thioglycolate broth. Genomic DNA was isolated (4) and sequenced using the Thermo Fisher Ion GeneStudio S5 system. Raw single-end reads were trimmed and processed using BBDuk tools v38.75 (<https://jgi.doe.gov/data-and-tools/bbtools/>). A total of 4,401,816 reads were obtained with an average length of 191 bp. Genomes were assembled using SPAdes v3.14.0 (5), assessed for completeness and contamination using CheckM v1.1.2 (6), and annotated using the NCBI Prokaryotic Genome Annotation Pipeline v4.12 (7). Default parameters were used for all software unless otherwise specified.

*E. callanderi* AMC0717 has a genome size of 4,327,770 bp, split across 85 contigs. There are 4,539 coding sequences and 54 RNA genes, with an overall GC content of 47.5%. AMC0717 shares 98.6% average nucleotide identity (ANI) (8) with the type strain, *Eubacterium callanderi* FD (2), and 93.9% with *Eubacterium limosum* ATCC 8486, the type species for the genus *Eubacterium*. No plasmids were identified using PlasmidFinder v2.0.1 (9). Phaster (10) identified one intact bacteriophage, a 36.6-kb region most similar to the *Clostridium* phage phiCD111 (11).

CARD v3.0.7 (12) identified a gene with 79% identity with an elfamycin-resistant EF-Tu gene, previously linked to pulvomycin resistance in *Escherichia coli*. Genes potentially encoding resistance to heavy metals, including mercury, copper, cobalt, zinc, and cadmium, were identified. One gene was identified encoding a toxin of the TlyA family RNA methyltransferase (i.e., a hemolysin). *Eubacterium callanderi* is considered nonmotile; however, a type IV pilus twitching motility protein, PilT (13), was identified in the genome but was not visible when viewed under scanning electron microscopy (Fig. 1). The genome contains a number of genes for exopolysaccharide (EPS) and capsule

**Citation** Marsh AJ, Chandrashekar K, Ng S, Roach J, Magness ST, Azcarate-Peril MA. 2020. Genome sequence of *Eubacterium callanderi* AMC0717, isolated from the colonic mucosa of an 11-year-old organ donor. *Microbiol Resour Announc* 9:e00995-20. <https://doi.org/10.1128/MRA.00995-20>.

**Editor** David Rasko, University of Maryland School of Medicine

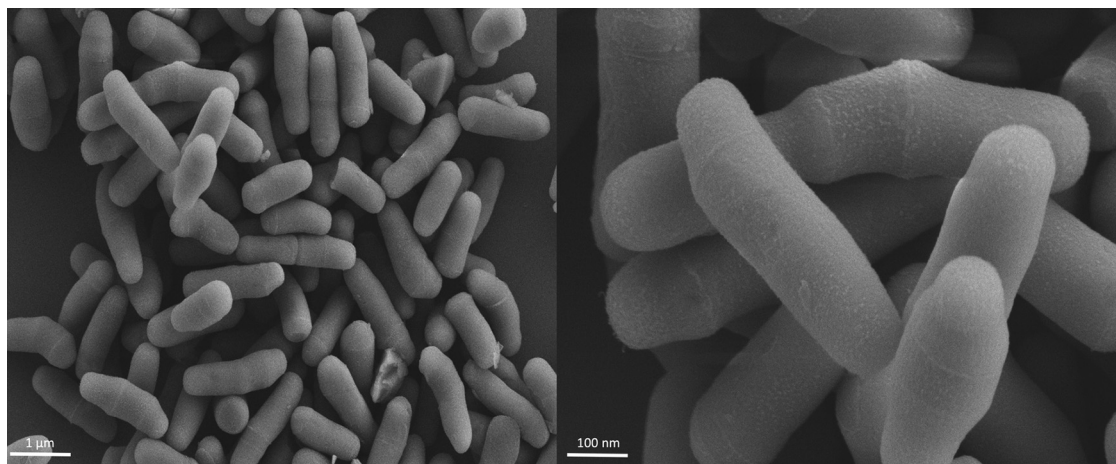
**Copyright** © 2020 Marsh et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to M. Andrea Azcarate-Peril, [azcarate@med.unc.edu](mailto:azcarate@med.unc.edu).

**Received** 8 September 2020

**Accepted** 25 October 2020

**Published** 12 November 2020



**FIG 1** Scanning electron microscope (SEM) imaging of *Eubacterium callanderi* AMC0717. Briefly, bacterial cell pellets were resuspended in 2% paraformaldehyde/2.5% glutaraldehyde in 0.15M sodium phosphate buffer, pH 7.4. Following treatment as described previously (17), the fixed cell suspension was deposited onto 12-mm round poly-D-lysine-coated coverslips, which, following preparation, were mounted onto 13-mm aluminum stubs and sputter coated with 5 nm of a gold-palladium alloy. A Zeiss Supra 25 field emission (FE) SEM operating at 5 kV was used to view the AMC0717 isolate with scanning electron microscopy.

production, including rhamnose-containing glycans. Putative gene clusters were present for the biosynthesis of three B vitamins, biotin (B7; *bioABDFW*), pyridoxine (B6; *pdxABHFJK*), and folate (B9; *folBEKB*), but further work is required to confirm the functionality of these genes. Homologs to two ribosomally synthesized, posttranslationally modified antimicrobial peptides (sactipeptide and linaridin) were identified using BAGEL4 (14), while gene homologs to terpene and another nonribosomal peptide synthetase were detected with AntiSMASH v5.1 (15). Putative genes belonging to the pyruvate-formate system were found in AMC0717, in addition to homologs for butanol biosynthesis and lactate, acetoin, and butanediol metabolism.

While previously of interest for the production of bioenergy (2, 16), the presence of genes for the synthesis of short-chain fatty acids (SCFAs) and vitamins indicates that AMC0717 may play an important role in gut health.

**Data availability.** This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank and SRA under the accession numbers [JACCKS000000000](https://www.ncbi.nlm.nih.gov/nuccore/JACCKS000000000) and [SRR12606937](https://www.ncbi.nlm.nih.gov/nuccore/SRR12606937), respectively. Additional information can be found at the AMC Culture Collection ([https://redcap.unc.edu/solutions/microbiome\\_core\\_986.php](https://redcap.unc.edu/solutions/microbiome_core_986.php)).

## ACKNOWLEDGMENTS

We thank the donors, their families, donor, her family, and Carolina Donor Services, an organ procurement organization in Durham, NC, for providing the samples used in this study. We are grateful to Kristen White and the Microscopy Services Laboratory, Department of Pathology and Laboratory Medicine, for their assistance with the SEM.

The UNC Microbiome Core is supported in part by the Center for Gastrointestinal Biology and Disease (CGIBD; P30 DK034987) and the UNC Nutrition Obesity Research Center (NORC; P30 DK056350). This work was supported by the UNC Core Facilities Advocacy Committee (CFAC). The Microscopy Services Laboratory is supported in part by Cancer Center Core Support Grant P30 CA016086 to the UNC Lineberger Comprehensive Cancer Center.

## REFERENCES

- Wade WG. 2006. The genus *Eubacterium* and related genera, p 823–835. In Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (ed), *The prokaryotes*. Springer, New York, NY.
- Mountfort DO, Grant WD, Clarke R, Asher RA. 1988. *Eubacterium callanderi* sp. nov. that demethoxylates O-methoxylated aromatic acids to volatile fatty acids. *Int J Syst Evol Microbiol* 38:254–258. <https://doi.org/10.1099/00207713-38-3-254>.
- Thiolas A, Bollet C, Gasmi M, Drancourt M, Raoult D. 2003. *Eubacterium*

- callanderi bacteremia: report of the first case. *J Clin Microbiol* 41:2235–2236. <https://doi.org/10.1128/jcm.41.5.2235-2236.2003>.
- Allali I, Arnold JW, Roach J, Cadenas MB, Butz N, Hassan HM, Koci M, Ballou A, Mendoza M, Ali R, Azcarate-Peril MA. 2017. A comparison of sequencing platforms and bioinformatics pipelines for compositional analysis of the gut microbiome. *BMC Microbiol* 17:194. <https://doi.org/10.1186/s12866-017-1101-8>.
  - 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>.
  - Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <https://doi.org/10.1101/gr.186072.114>.
  - 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>.
  - Yoon S-H, Ha S-M, 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>.
  - Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, Moller Aarestrup F, Hasman H. 2014. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
  - Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 44:W16–W21. <https://doi.org/10.1093/nar/gkw387>.
  - Gameau JR, Depardieu F, Fortier L-C, Bikard D, Monot M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. *Sci Rep* 7:8292. <https://doi.org/10.1038/s41598-017-07910-5>.
  - Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Res* 45:D566–D573. <https://doi.org/10.1093/nar/gkw1004>.
  - Piepenbrink KH, Sundberg EJ. 2016. Motility and adhesion through type IV pili in Gram-positive bacteria. *Biochem Soc Trans* 44:1659–1666. <https://doi.org/10.1042/BST20160221>.
  - van Heel AJ, de Jong A, Song C, Viel JH, Kok J, Kuipers OP. 2018. BAGEL4: a user-friendly Web server to thoroughly mine RiPPs and bacteriocins. *Nucleic Acids Res* 46:W278–W281. <https://doi.org/10.1093/nar/gky383>.
  - Blin K, Pascal Andreu V, de Los Santos ELC, Del Carratore F, Lee SY, Medema MH, Weber T. 2019. The antiSMASH database version 2: a comprehensive resource on secondary metabolite biosynthetic gene clusters. *Nucleic Acids Res* 47:D625–D630. <https://doi.org/10.1093/nar/gky1060>.
  - Roh H, Ko H-J, Kim D, Choi DG, Park S, Kim S, Chang IS, Choi I-G. 2011. Complete genome sequence of a carbon monoxide-utilizing acetogen, *Eubacterium limosum* KIST612. *J Bacteriol* 193:307–308. <https://doi.org/10.1128/JB.01217-10>.
  - Katsumoto T, Naguro T, Iino A, Takagi A. 1981. The effect of tannic acid on the preservation of tissue culture cells for scanning electron microscopy. *J Electron Microscop* (Tokyo) 30:177–182. <https://doi.org/10.1093/oxfordjournals/jmicro.a050303>.