




Genome Sequence of the Fish Brain Bacterium *Clostridium tarantellae*

Luca Bano,^a Matthias Kiel,^b Gabriele Sales,^c Andrew C. Doxey,^d Michael J. Mansfield,^d Haleluya T. Wami,^b Marco Schiavone,^e Ornella Rossetto,^e Marco Pirazzini,^e  Ulrich Dobrindt,^b Cesare Montecucco^{b,f}

^aMicrobiology Laboratory, Istituto Zooprofilattico Sperimentale delle Venezie, Villorba, Italy

^bInstitute of Hygiene, University of Muenster, Muenster, Germany

^cDepartment of Biology, University of Padua, Padua, Italy

^dDepartment of Biology, University of Waterloo, Waterloo, Canada

^eDepartment of Biomedical Sciences, University of Padua, Padua, Italy

^fCNR Institute of Neuroscience, Padua, Italy

ABSTRACT *Eubacterium tarantellae* was originally cultivated from the brain of fish affected by twirling movements. Here, we present the draft genome sequence of *E. tarantellae* DSM 3997, which consists of 3,982,316 bp. Most protein-coding genes in this strain are similar to genes of *Clostridium* bacteria, supporting the renaming of *E. tarantellae* as *Clostridium tarantellae*.

The brain is generally considered a bacterium-free organ that can be infected only after alteration of the blood-brain barrier. However, Udey et al. (1) reported the unique isolation of a bacterium from the brain of apparently healthy fish displaying a rapid turning-around movement (twirling). This behavioral change is most likely to be the consequence of an alteration of the brain circuits that control body movements. Accordingly, this bacterium was named *Eubacterium tarantellae*, described as a non-spore-forming anaerobe and suggested to be the first of a novel class of anaerobic fish pathogenic bacteria (2). No further characterization was reported, though it was isolated from fish larval gut flora (3) and recently associated with a human case of joint septic arthritis (4).

E. tarantellae DSM 3997 was cultivated in synthetic thioglycolate broth under anaerobiosis. DNA was automatically extracted (MicroLab STARlet; Hamilton, Switzerland) using a MagMAX total nucleic acid isolation kit (Ambion/Life Technologies, USA). Whole-genome sequencing was performed using an Illumina MiSeq sequencing platform. To prepare 500-bp paired-end libraries of *E. tarantellae*, we used the Nextera XT DNA library preparation kit (Illumina, USA). Libraries were sequenced using V2 sequencing chemistry. Quality trimming of 2,896,670 raw reads (1,448,335 pairs) was performed using Sickle v1.33 (<https://github.com/najoshi/sickle>) with the options `-l 30` and `-q 28` and resulted in 2,213,220 single reads corresponding to 1,106,610 paired-end reads. *De novo* assembly of the processed reads was performed with SPAdes v3.10.1 using default parameters (5). The assembly resulted in 713 contigs, validated using QualiMap v2.1, with an N_{50} contig size of 11,440 bp. The draft genome consists of 3,979,886 bp with a 25.8% GC content and an average coverage of 149 \times . The genome assembly was estimated to be 98.5% complete using CheckM (6) with only 5% redundancy.

Automatic gene prediction was performed using PGAP v4.10 (www.ncbi.nlm.nih.gov/genome/annotation_prok/) (7). The draft genome encodes 3,069 predicted protein-coding genes, including 2,274 genes with a predicted function and 795 genes coding for hypothetical proteins. Additionally, 11 rRNA and 72 tRNA genes were predicted. Furthermore, one contig includes a clustered regularly interspaced short

Citation Bano L, Kiel M, Sales G, Doxey AC, Mansfield MJ, Wami HT, Schiavone M, Rossetto O, Pirazzini M, Dobrindt U, Montecucco C. 2020. Genome sequence of the fish brain bacterium *Clostridium tarantellae*. *Microbiol Resour Announc* 9:e01575-19. <https://doi.org/10.1128/MRA.01575-19>.

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

Copyright © 2020 Bano 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 Ulrich Dobrindt, dobrindt@uni-muenster.de, or Cesare Montecucco, cesare.montecucco@gmail.com.

Received 23 December 2019

Accepted 26 March 2020

Published 16 April 2020

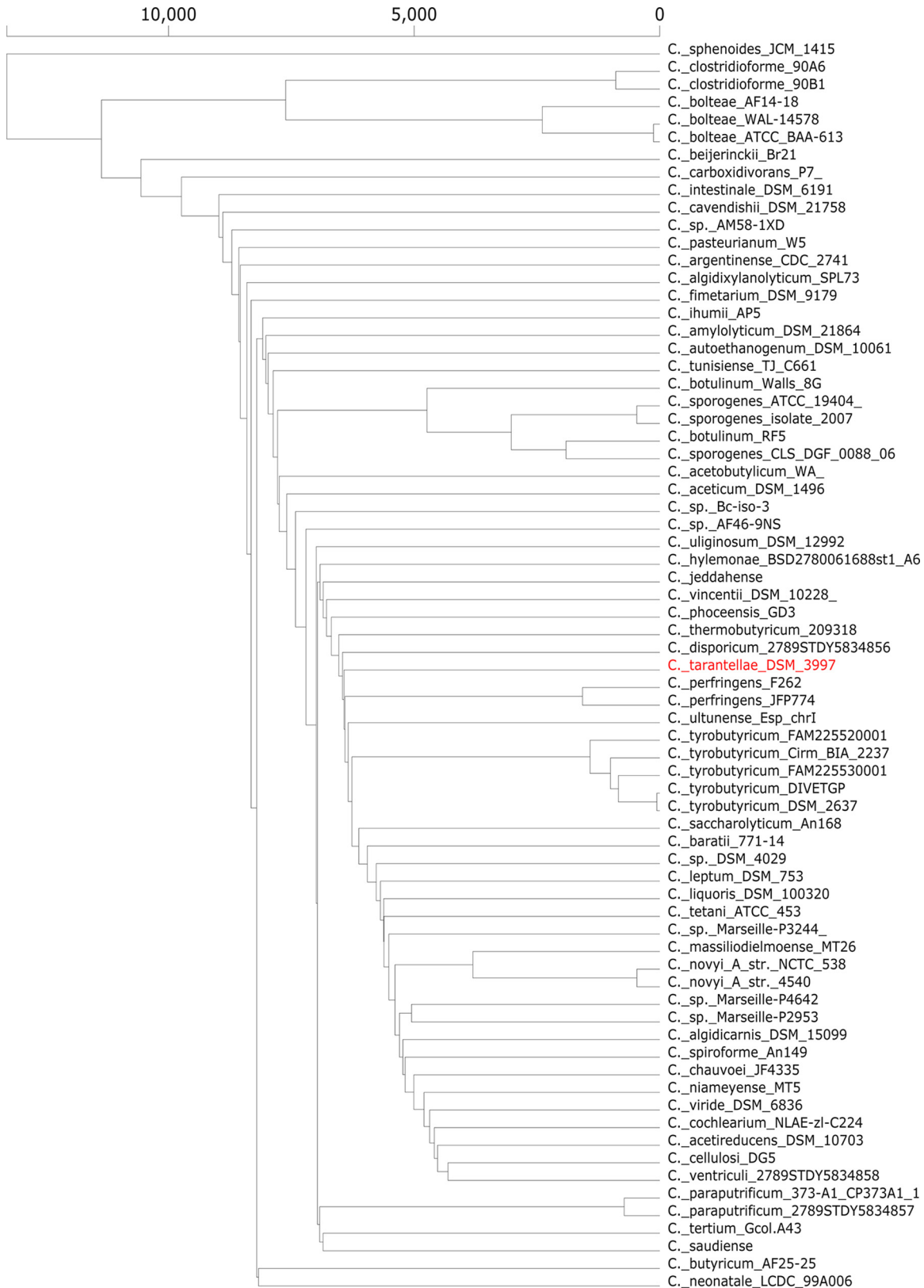


FIG 1 Comparison of the genome content of clostridial species based on the presence/absence of genes. The pan-genome presence/absence analysis of 71 *Clostridium* strains was performed using Roary v3.12.0 with default parameters. The generated tree was visualized using FriPan (<https://github.com/drpowell/FriPan>).

Downloaded from <http://mra.asm.org/> on May 2, 2020 by guest

palindromic repeat (CRISPR)-associated (Cas) operon including putative genes homologous to Cas3, Cas5, Cas7, and Cas8b/Csh1 from other clostridia.

Phylogenetic analysis of taxonomic markers (8) showed that the *E. tarantellae* genome is closely related to that of *Clostridium perfringens*, followed by those of other *Clostridium* species. Pan-genome analysis based on the presence/absence of genes in publicly available clostridial genome sequences and in the *C. tarantellae* DSM 3997 genome was performed with Roary v3.12.0 (8) with default parameters and corroborated that *C. perfringens* and *C. tarantellae* are indeed closely related (Fig. 1). Due to the peculiar association between *E. tarantellae* and fish brain/movement alteration, we searched the genome for potential neurotoxins possibly responsible for inducing twirling using the virulence factor database (VFDB) (9). No genes related to clostridial neurotoxins were found. However, a gene encoding α -perfringolysin-like pore-forming toxin from *C. perfringens* not known to induce alteration of movements is present. In addition, several putative toxins similar to the *Clostridium septicum* epsilon toxin ETX were identified. Furthermore, three leukocidins/hemolysins similar to the beta-channel-forming toxin NetE of *C. perfringens* and some clostridial-type phospholipases were predicted to be encoded in the *E. tarantellae* genome. Small cysteine-rich proteins that encode possible antimicrobial peptides or peptide toxins were also identified (e.g., CEFHDAMA_03104 and CEFHDAMA_00788) and found to be conserved in other clostridia. In addition, though *E. tarantellae* was not found to sporulate (1, 2), the genes encoding proteins involved in the execution of the sporogenesis program are present. The high similarity of the present genome to those of clostridial species strongly supports the suggestion that *Eubacterium tarantellae* should be renamed *Clostridium tarantellae* (10).

Data availability. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [WHJC00000000](https://www.ncbi.nlm.nih.gov/nuclseq/WHJC00000000). The version described in this paper is version WHJC01000000. The raw reads are available at the Sequence Read Archive under the accession number [PRJNA587720](https://www.ncbi.nlm.nih.gov/sra/PRJNA587720).

ACKNOWLEDGMENTS

We thank K. Tegelkamp (Münster) for excellent technical support.

This work was supported by the University of Padua, by the IZTV, and by the German Research Foundation (grants SFB 1009 and TP B05).

We thank the reviewers for their improvements to the paper.

REFERENCES

1. Udey L, Young RE, Sallman B. 1977. Isolation and characterization of an anaerobic bacterium, *Eubacterium tarantellus* sp.nov., associated with striped mullet (*Mugil cephalus*) mortality in Biscayne Bay, Florida. *J Fish Res Bd Can* 34:402–409. <https://doi.org/10.1139/f77-064>.
2. Udey LR. 1978. Anaerobic bacteria as possible disease agents in fish. *Marine Fish Rev* 40:10–12.
3. Akayli T, Erkan M, Yardimci RE, Ozgur C, Urku C. 2015. Interaction of gut flora and bacterial pathogens of cultured common dentex (*Dentex dentex*). *Isr J Aquacult*. <http://hdl.handle.net/10524/49173>.
4. Cointe A, de Ponfily GP, Munier AL, Bachir M, Benmansour H, Crémieux AC, Forien M, Frazier A, Krief E, Cambau E, Jacquier H. 2019. Native joint septic arthritis due to *Clostridium tarantellae*. *Anaerobe* 56:46–48. <https://doi.org/10.1016/j.anaerobe.2019.02.004>.
5. 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>.
6. 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>.
7. Petrenko P, Lobb B, Kurtz DA, Neufeld JD, Doxey AC. 2015. MetAnnotate: function-specific taxonomic profiling and comparison of metagenomes. *BMC Biol* 13:92. <https://doi.org/10.1186/s12915-015-0195-4>.
8. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, Fookes M, Falush D, Keane JA, Parkhill J. 2015. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31:3691–3693. <https://doi.org/10.1093/bioinformatics/btv421>.
9. 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>.
10. Lawson PA, Rainey FA. 2016. Proposal to restrict the genus *Clostridium* *Prazmowski* to *Clostridium butyricum* and related species. *Int J Syst Evol Microbiol* 66:1009–1016. <https://doi.org/10.1099/ijsem.0.000824>.