



Complete Genome Sequence of a New *Bacteroidaceae* Bacterium Isolated from Anaerobic Biomass Digestion

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ABSTRACT Here, we present the genome sequence and annotation of HV4-6-C5C, a bacterial strain isolated from a mesophilic two-stage laboratory-scale leach bed biogas reactor system. Strain HV4-6-C5C may represent a new genus of the family *Bacteroidaceae* and may have a key role in acidogenesis and acetogenesis steps during anaerobic biomass digestion.

A new bacterial strain, named HV4-6-C5C, was isolated from a mesophilic laboratory-scale leach bed system using freshly cut grass as the substrate (coordinates 50°51'55.4"N, 11°35'56.1"E). For microbial isolation, BBL Columbia agar (BD Biosciences) supplemented with 5% laked horse blood was applied. DNA was extracted and purified using the Gentra Puregene Yeast/Bact. kit (Qiagen) and the NucleoSpin genomic DNA (gDNA) clean-up kit (Macherey-Nagel). We constructed a Nextera XT library from total genomic DNA and sequenced it using the Illumina NextSeq 500 platform (150-bp paired-end reads). Raw reads were filtered (quality score [Q], >20; minimum length, >50 nucleotides [nt]) with BBTools v37.10, yielding 26.42 million paired-end sequences with a mean Q value of 33.24. Genome assembly was conducted with SPAdes v3.13.0 (1). A total of 63 contigs with a length of ≥ 300 nt were obtained, covering a total genome size of 3,869,825 nt and with an estimated GC content of 39.30%. The largest contig was 424,839 nt, and the N_{50} value was 183,010 nt.

The assembled sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (2) and the Rapid Annotations using Subsystems Technology toolkit (RASTtk) (3) implemented in the Pathosystems Resource Integration Center (PATRIC) system (4). The genome of strain HV4-6-C5C harbors 2,990 genes, including 2,934 open reading frames, 68 pseudogenes, 3 rRNAs organized in a single operon, 51 tRNAs, and 2 noncoding RNAs (ncRNAs). The genome encodes the enzymes for methylmalonyl-coenzyme A (CoA) mutase (EC 5.4.99.2) and methylmalonyl-CoA epimerase (EC 5.1.99.1), two key enzymes involved in the fermentation of lactate via methylmalonyl-CoA to acetate plus propionate. In addition, the enzymes necessary to perform the conversion of acetyl-CoA to acetate, namely, acetate kinase (EC 2.7.2.1) and phosphate acetyltransferase (EC 2.3.1.8), were also identified. Therefore, strain HV4-6-C5C may have a key role in the acidogenesis and acetogenesis steps during the anaerobic digestion of organic matter.

Citation Hahnke S, Abendroth C, Pascual J, Langer T, Codoñer FM, Ramm P, Klocke M, Luschniq O, Porcar M. 2019. Complete genome sequence of a new *Bacteroidaceae* bacterium isolated from anaerobic biomass digestion. *Microbiol Resour Announc* 8:e01203-19. <https://doi.org/10.1128/MRA.01203-19>.

Editor Julie C. Dunning Hotopp, University of Maryland School of Medicine

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Received 29 September 2019

Accepted 24 October 2019

Published 14 November 2019

In order to investigate the taxonomic affiliation of strain HV4-6-C5C, the full-length 16S rRNA gene sequence was compared against the online EzBioCloud database (5). The type strain closest to HV4-6-C5C was *Bacteroides stercorisoris* JCM 17103, which shared 94.04% gene sequence similarity with HV4-6-C5C. This result, along with the low average nucleotide identity (6) value between strain HV4-6-C5C and its closest type strains (<95.0%) confirmed that strain HV4-6-C5C is a novel species (7). The average amino acid identity (8) value calculated for strain HV4-6-C5C and *B. fragilis* NCTC 9343, the type species of the genus *Bacteroides*, was 67.3%, a value close to the threshold for definition of a new genus (8). However, the percentage of conserved proteins (POCP) (9) and the 16S rRNA gene sequence similarity values for strain HV4-6-C5C and *B. fragilis* NCTC 9343^T were 48.4% and 91.3%, respectively. Both values were lower than the thresholds for the definition of a new genus (9, 10). Hence, we can conclude that strain HV4-6-C5C may represent a new genus within the family *Bacteroidaceae*.

Data availability. Strain HV4-6-C5C was deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) under the strain designation DSM 104071. This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number [VOIU00000000](https://www.ncbi.nlm.nih.gov/nuclink/VOIU00000000). The version described in this paper is the first version, VOIU01000000. Raw sequence reads are deposited under accession number [SRR10177305](https://www.ncbi.nlm.nih.gov/nuclink/SRR10177305). The WGS and SRA records are associated with BioProject number [PRJNA557591](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA557591).

ACKNOWLEDGMENTS

We acknowledge funding by the European Union through the BioRoboost project (H2020-NMBP-TR-IND-2018-2020/BIOTEC-01-2018 [Coordination and Support Action], project identifier 210491758). Moreover, this study was funded by the German Federal Ministry of Economic Affairs and Energy (grant numbers KF 2050830SA4, KF 3400701SA4, and KF 2112205SA4).

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