



Draft Genome Sequence of a New *Oscillospiraceae* Bacterium Isolated from Anaerobic Digestion of Biomass

Javier Pascual,^a Sarah Hahnke,^{b,c}  Christian Abendroth,^{d,e} Thomas Langer,^b Patrice Ramm,^{b,f} Michael Klocke,^f Olaf Luschnig,^g Manuel Porcar^{a,h}

^aDarwin Bioprospecting Excellence, S.L., Paterna, Valencia, Spain

^bLeibniz Institute for Agricultural Engineering and Bioeconomy e.V. (ATB), Department Bioengineering, Potsdam, Germany

^cUniversity of Oldenburg, Department of Human Medicine, Oldenburg, Germany

^dRobert Boyle Institut e.V., Jena, Germany

^eInstitute of Waste Management and Circular Economy, Technische Universität Dresden, Pirna, Germany

^fInstitute of Agricultural and Urban Ecological Projects affiliated to Berlin Humboldt University (IASP), Berlin, Germany

^gBio H2 Umwelt GmbH, Jena, Germany

^hInstitute for Integrative Systems Biology (I2SysBio), University of Valencia-CSIC, Paterna, Valencia, Spain

ABSTRACT Here, we present the genome sequence and annotation of the novel bacterial strain HV4-5-C5C, which may represent a new genus within the family *Oscillospiraceae* (order *Eubacteriales*). This strain is a potential keystone species in the hydrolysis of complex polymers during anaerobic digestion of biomass.

In the past few years, efforts have been undertaken to characterize new species isolated from anaerobic digesters (1, 2). However, few articles have focused on microorganisms isolated from acidic pretreatment stages. We present here the draft genome sequence of the bacterial strain HV4-5-C5C, which was isolated from the acidification stage of a mesophilic two-stage laboratory-scale leach bed system using as the sole substrate freshly cut grass taken from a meadow in Jena, Germany (50°51'55.4"N, 11°35'56.1"E). Isolation of the strain was performed after the diluted hydrolysate was reincubated with microcrystalline cellulose as the sole carbon source. After incubation, the hydrolysate was diluted 10⁵-fold, plated on BBL Columbia agar (BD Biosciences) supplemented with 5% laked horse blood, and cultivated under anoxic conditions at 37°C. For purification, single colonies were picked and restreaked several times.

After cultivation in brain heart infusion broth (Carl Roth) supplemented with yeast extract, DNA was extracted and purified using the Genra Puregene Yeast/Bact. kit (Qiagen) and the NucleoSpin genomic DNA (gDNA) cleanup kit (Macherey-Nagel). We constructed a Nextera XT library from the total genomic DNA and sequenced it using the Illumina NextSeq 500 platform (150-bp paired-end reads). The raw reads were filtered (quality [Q], >20; minimum length, >50 nucleotides [nt]) with BBTools v37.10, yielding 23.48 million paired-end sequences with a mean Q value of 32.93. Genome assembly was conducted with the software SPAdes v3.13.0 (3). A total of 72 contigs were obtained (length, ≥300 nt), covering a total genome size of 2,867,854 nt with an estimated GC content of 53.25%. The largest contig was 296,629 nt, and the N_{50} value was 134,989 nt. The final coverage of the genome was 2,457×.

The assembled sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (4) and the RAST toolkit (5) implemented in PATRIC (6). The genome of strain HV4-5-C5C harbors 2,462 genes, including 2,364 coding DNA sequences (CDSs), 45 pseudogenes, 3 rRNAs organized in a single operon, 47 tRNAs, and 3 noncoding RNAs (ncRNAs).

Citation Pascual J, Hahnke S, Abendroth C, Langer T, Ramm P, Klocke M, Luschnig O, Porcar M. 2020. Draft genome sequence of a new *Oscillospiraceae* bacterium isolated from anaerobic digestion of biomass. *Microbiol Resour Announc* 9:e00507-20. <https://doi.org/10.1128/MRA.00507-20>.

Editor J. Cameron Thrash, University of Southern California

Copyright © 2020 Pascual 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 Christian Abendroth, christian.abendroth@tu-dresden.de, or Manuel Porcar, manuel.porcar@uv.es.

Received 2 May 2020

Accepted 15 June 2020

Published 2 July 2020

TABLE 1 Metabolic pathways involved in the acidogenesis and acetogenesis steps

Metabolic pathway	Enzyme(s) (EC no.)
Alcoholic fermentation	Ethanol dehydrogenase (1.1.1.1)
Formation of lactate	L- and D-lactate dehydrogenases (1.1.1.27; 1.1.1.28)
Conversion of pyruvate to acetyl-CoA ^a and formate	Pyruvate formate lyase (2.3.1.54)
Conversion of acetyl-CoA to acetate	Acetate kinase (2.7.2.1) and phosphate acetyltransferase (2.3.1.8)

^a CoA, coenzyme A.

The genome harbors 88 different glycoside hydrolases and 2 polysaccharide lyases (7). Strain HV4-5-C5C might be able to carry out alcoholic fermentation as well as the synthesis of lactate, formate, and acetate (Table 1). Therefore, strain HV4-5-C5C may be a keystone species in the hydrolysis of complex polymers as well as in the acidogenesis and acetogenesis steps.

The type strain most closely related to HV4-5-C5C was *Mageeibacillus indolicus* CCUG 59143, sharing 90.31% 16S rRNA gene sequence similarity (EzBioCloud, v2020.02.25 [8]). Calculation of the average nucleotide identity (JSpecies tool v3.4.7) resulted in a value of 65.10% between both strains (9, 10).

The average amino acid identity (11) and the percentage of conserved proteins (12) calculated for strain HV4-5-C5C and *M. indolicus* CCUG 59143, the type species of the genus *Mageeibacillus*, were 15.16% and 25.13%, respectively. Hence, we can assume that strain HV4-5-C5C may represent a new genus within the family *Oscillospiraceae* (order *Eubacteriales*) (11, 12). Default parameters were used for all software unless otherwise specified.

Data availability. Strain HV4-5-C5C was deposited at the German Collection of Microorganisms and Cell Cultures under the designation DSM 103941. This whole-genome sequencing (WGS) project has been deposited at DDBJ/ENA/GenBank under the accession number [JAAVLZ000000000.1](https://www.ncbi.nlm.nih.gov/assembly/JAAVLZ000000000.1). The raw sequence reads are deposited under SRA accession number [SRR11413021](https://www.ncbi.nlm.nih.gov/sra/SRR11413021). The WGS and SRA records are associated with BioProject accession number [PRJNA614915](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA614915).

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 ID 210491758. This study was also funded by the German Federal Ministry of Economic Affairs and Energy (grant numbers KF 2050830SA4, KF 3400701SA4, and KF 2112205SA4). Finally, we are grateful for open access funding by the publication fund of the Technische Universität Dresden.

REFERENCES

- Kirkegaard RH, McIlroy SJ, Kristensen JM, Nierychlo M, Karst SM, Dueholm MS, Albertsen M, Nielsen PH. 2017. The impact of immigration on microbial community composition in full-scale anaerobic digesters. *Sci Rep* 7:9343. <https://doi.org/10.1038/s41598-017-09303-0>.
- Campanaro S, Treu L, Rodriguez-R LM, Kovalovszki A, Ziels RM, Maus I, Zhu X, Kougias PG, Basile A, Luo G, Schlüter A, Konstantinidis KT, Angelidaki I. 2020. New insights from the biogas microbiome by comprehensive genome-resolved metagenomics of nearly 1600 species originating from multiple anaerobic digesters. *Biotechnol Biofuels* 13:25. <https://doi.org/10.1186/s13068-020-01679-y>.
- 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>.
- 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* 44:6614–2664. <https://doi.org/10.1093/nar/gkw569>.
- Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, III, 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>.
- Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45:D535–D542. <https://doi.org/10.1093/nar/gkw1017>.
- Lombard V, Golaconda Ramulu H, Drula E, Coutinho PM, Henrissat B. 2014. The Carbohydrate-Active Enzymes database (CAZy) in 2013. *Nucleic Acids Res* 42:D490–D495. <https://doi.org/10.1093/nar/gkt1178>.
- 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 and whole genome assemblies. *Int J Syst Evol Microbiol* 67:1613–1617. <https://doi.org/10.1099/ijsem.0.001755>.

9. Richter M, Rosselló-Móra R, Glöckner FO, Peplies J. 2016. JSpeciesWS: a Web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>.
10. Richter M, Rosselló-Móra R. 2009. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci U S A* 106: 19126–19131. <https://doi.org/10.1073/pnas.0906412106>.
11. Rodriguez-R LM, Konstantinidis KT. 2016. The enveomics collection: a toolbox for specialized analyses of microbial genomes and metagenomes. *PeerJ Prepr* <https://peerj.com/preprints/1900/>.
12. Qin Q-L, Xie B-B, Zhang X-Y, Chen X-L, Zhou B-C, Zhou J, Oren A, Zhang Y-Z. 2014. A proposed genus boundary for the prokaryotes based on genomic insights. *J Bacteriol* 196:2210–2215. <https://doi.org/10.1128/JB.01688-14>.