



# Complete Genome Sequence of a New *Ruminococcaceae* Bacterium Isolated from Anaerobic Biomass Hydrolysis

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**ABSTRACT** A new *Ruminococcaceae* bacterium, strain HV4-5-B5C, participating in the anaerobic digestion of grass, was isolated from a mesophilic two-stage laboratory-scale leach bed biogas system. The draft annotated genome sequence presented in this study and 16S rRNA gene sequence analysis indicated the affiliation of HV4-5-B5C with the family *Ruminococcaceae* outside recently described genera.

**A**naerobic digestion is a promising technology to generate biofuels and other products from biomass (1). However, depending on the lignocellulose content of the biomass, the degradation rate varies. Hence, a physical or biological biomass pretreatment can be helpful to support the microbial hydrolysis (2).

In order to enrich hydrolytic bacteria, a mesophilic (37°C) two-stage laboratory-scale leach bed system for biomethanation of freshly cut grass as the sole substrate was set up. From the process fluid, a new *Ruminococcaceae* bacterium, strain HV4-5-B5C, was isolated, presumably participating in the degradation of plant biomass. Isolation was performed under anoxic conditions on Anaerobic agar acc. to Brewer (Merck) after the diluted hydrolysate had been reincubated with microcrystalline cellulose as the sole carbon source.

Genome sequencing was performed using the Illumina NextSeq 500 platform. A Nextera XT library with a mean insert size of around 300 nucleotides (nt) was constructed and sequenced in a combination of 150-bp paired-end (PE) reads. In total, 21,629,863 PE sequences with a mean length of 150.32 nt were obtained. After quality filtering, 21.61 million PE sequences remained, with a mean Q value of 32.71. Genome assembly was conducted with software SPAdes version 3.10.1 (3) and default parameters using a *k*-mer of 127. A total of 5,019 contigs were obtained, including 76 contigs with a length of over 500 nt covering a total genome size of around 3.01 Mb, with an estimated GC content of 52.50%. The longest calculated contig was 581,597 nt, and the *N*<sub>50</sub> value of the assembly was 360,256 nt.

The assembled sequences were annotated using the Prokka annotation pipeline version 1.11, including the prediction of tRNA, rRNA, and mRNA genes and signal peptides using the Aragorn, RNAmmer, Prodigal, and SignalP software (4–8).

The genome of strain HV4-5-B5C contained 2,945 elements, of which 2,878 were open reading frames (ORFs), with 2,325 canonical ORFs and 553 noncanonical ORFs, and 67 elements that encoded structural RNAs (sRNAs), with 6 for rRNA and 61 for tRNA.

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Using BLAST, the determined contigs were compared with all genome sequences available at the NCBI database of complete bacterial genomes. Based on the percentage of conserved proteins (POCP) (9), the closest genome-sequenced relative of the novel *Ruminococcaceae* bacterium was *Clostridium sporosphaeroides* (38.31% identity), as also indicated by the average amino acid identity (AAI) (10) (54.72% identity). Analysis based on the average nucleotide sequence identity (ANI) (11) showed closest affiliation to *Clostridium sporosphaeroides* (68.83% identity), applying ANIBlast, and to *Clostridium leptum* (88.36% identity), applying ANI-MUMmer. Using the EzBioCloud identifier (12), 16S rRNA gene sequence comparisons revealed *Caproiciproducens galactitolivorans* BS-1<sup>T</sup> to be the most closely affiliated strain, sharing 93.3% sequence identity. In summary, these results indicate that the novel bacterial strain represents a new species and possibly a new genus within the family *Ruminococcaceae*.

**Accession number(s).** Strain HV4-5-B5C was deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) under accession no. DSM-104463. The genome project was deposited at DDBJ/EMBL/GenBank under accession no. [FXYJ02000001](#) to [FXYJ02000076](#). The version described here is the first draft version.

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## REFERENCES

- Strong PJ, Kalyuzhnaya M, Silverman J, Clarke WP. 2016. A methanotroph-based biorefinery: potential scenarios for generating multiple products from a single fermentation. *Bioresour Technol* 215:314–323. <https://doi.org/10.1016/j.biortech.2016.04.099>.
- Paudel SR, Banjara SP, Choi OK, Park KY, Kim YM, Lee JW. 2017. Pretreatment of agricultural biomass for anaerobic digestion: current state and challenges. *Bioresour Technol* 245:1194–1205. <https://doi.org/10.1016/j.biortech.2017.08.182>.
- Bankovich 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>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res* 32:11–16. <https://doi.org/10.1093/nar/gkh152>.
- Lagesen K, Hallin P, Rødland EA, Stærfeldt H-H, Rognes T, Ussery DW. 2007. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 35:3100–3108. <https://doi.org/10.1093/nar/gkm160>.
- Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>.
- Petersen TN, Brunak S, von Heijne G, Nielsen H. 2011. SignalP 4.0: discriminating signal peptides from transmembrane regions. *Nat Methods* 8:785–786. <https://doi.org/10.1038/nmeth.1701>.
- Qin QL, Xie BB, Zhang XY, Chen XL, Zhou BC, Zhou J, Oren A, Zhang YZ. 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>.
- Konstantinidis KT, Tiedje JM. 2005. Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* 187:6258–6264. <https://doi.org/10.1128/JB.187.18.6258-6264.2005>.
- Konstantinidis KT, Tiedje JM. 2005. Genomic insights that advance the species definition for prokaryotes. *Proc Natl Acad Sci U S A* 102:2567–2572. <https://doi.org/10.1073/pnas.0409727102>.
- Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J. 2012. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 62:716–721. <https://doi.org/10.1099/ijs.0.038075-0>.