



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

Sarah Hahnke,<sup>a</sup> Christian Abendroth,<sup>b,c,d,e</sup> Thomas Langer,<sup>a</sup> Francisco M. Codoñer,<sup>f</sup> Patrice Ramm,<sup>a</sup> Manuel Porcar,<sup>b,c,g</sup> Olaf Luschig,<sup>h</sup> Michael Klocke<sup>a</sup>

<sup>a</sup>Department of Bioengineering, Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB), Potsdam, Germany

<sup>b</sup>Cavanilles Institute of Biodiversity and Evolutionary Biology, Universitat de Valencia, Paterna, Valencia, Spain

<sup>c</sup>Institute for Integrative Systems Biology (I<sup>2</sup>SysBio), Paterna, Valencia, Spain

<sup>d</sup>Robert Boyle Institut e.V., Jena, Germany

<sup>e</sup>Chair of Waste Management, Technische Universität Dresden, Pirna, Germany

<sup>f</sup>Lifesequencing SL, Parc Científic Universitat de Valencia, Paterna, Valencia, Spain

<sup>g</sup>Darwin Bioprospecting Excellence, S.L. Parc Científic Universitat de Valencia, Paterna, Valencia, Spain

<sup>h</sup>Bio H2 Energy GmbH, Jena, Germany

**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.

Anaerobic 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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Address correspondence to Michael Klocke, [mklocke@atb-potsdam.de](mailto:mklocke@atb-potsdam.de).

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](https://doi.org/10.1093/nar/gkx152) to [FXYJ02000076](https://doi.org/10.1093/nar/gkx152). The version described here is the first draft version.

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