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GENOME SEQUENCES

Full Genome Sequence of a *Methanomassiliicoccales* Representative Enriched from Peat Soil

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ABSTRACT The full genome of a *Methanomassiliicoccales* strain, U3.2.1, was obtained from enrichment cultures of percolation fen peat soil under methanogenic conditions, with methanol and hydrogen as the electron acceptor and donor, respectively. Metagenomic assembly of combined long-read and short-read sequences resulted in a 1.51-Mbp circular genome.

Methane from methanogenic archaea in wetland peat soils contributes 33% to the global biological methane emissions (1). The methanogenic order *Methanomassiliicoccales* (2, 3), which was discovered in 2012, was detected in wetland soils worldwide, sometimes representing a substantial fraction of the methanogenic community (4–6). To date, only a single isolate has been validly described, *Methanomassiliicoccus luminyensis* 10B, which was isolated from human feces (2). A draft genome of *M. luminyensis* and a few complete genomes of *Methanomassiliicoccales* enrichment cultures, obtained mainly from gastrointestinal tracts of animals and humans but also from the environment, have been described since then (4, 7–12). However, no highly enriched *Methanomassiliicoccales* cultures or complete genomes from peat soil have been obtained thus far. Here, we present the complete circular genome of a *Methanomassiliicoccales* member enriched from peat soil from a percolation fen, strain U3.2.1.

The peat used for enrichment was sampled in a percolation fen in northeastern Germany (54.10N, 12.74E), at a depth of 25 cm (13). Modified MpT1 medium, with methanol as the electron acceptor under an anaerobic H_2/CO_2 atmosphere, was used for the enrichment of *Methanomassiliicoccales* as described previously. Enrichment success was monitored with quantitative PCR (4). DNA was extracted with a phenol/chloroform-based DNA extraction protocol (14). Gene quantification was done by quantitative PCR, using the primer pairs AS1/AS2 and EuFor/EuRev for *Methanomassiliicoccales* and bacteria, respectively (15, 16). After several rounds of culture incubations for 2 weeks and consecutive transfers, 16S rRNA genes of *Methanomassiliicoccales* accounted for 60% of the total prokaryotic 16S rRNA genes. Paired-end-read Illumina MiSeq v4 sequencing was performed by LGC Genomics (Berlin, Germany) using the primer pair 515YF/B806R for prokaryotes (17). The raw sequence reads were processed by demultiplexing and removing barcodes, adapters, and primers using the Illumina bcl2fastq software. The data were then quality filtered with the DADA2 v1.8.0 pipeline (18) in R v3.5. The data revealed the presence of a single 16S rRNA gene sequence of *Methanomassiliicoccales* in several of the enrichment cultures.

Without DNA shearing and size selection, long-read sequencing libraries were prepared from enrichment culture U.3.2.1 using the ligation sequencing kit (SQK-LSK109; Oxford Nanopore Technologies) and then were sequenced on a MinION Mk1B sequencer (Oxford

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Nanopore Technologies) using the kit flow cell (R9.4.1), producing 3.43 million reads (12.2 Gb). Base calling for MinION reads was performed with Guppy v2.3.7 with the flip-flop model. For short-read sequencing, libraries were prepared from the same DNA extract with the NEBNext Ultra II DNA library preparation kit (New England Biolabs) and sequenced on a MiSeq platform with a MiSeq reagent microkit v2 (Illumina). MinION reads were quality trimmed and filtered with NanoFilt v2.3.0 (19) with parameters -l 2000 -q 7. This resulted in 3,299,097 reads, with an N_{50} value of 1,099,330 nucleotides. In the following steps, default parameters were used. An initial metagenome assembly was generated with MetaSPAdes v3.11.0 (20). MinION reads were mapped against the assembly using minimap2 v2.17-r943-dirty (21), and Illumina reads were mapped to the assembly with BWA v0.7.17-r1188 (22). Contigs of the initial assembly were searched for hits in the UniProt reference proteomes (23) (https://ftp.uniprot.org/pub/databases/uniprot/current_release/knowledgebase/complete/uniprot_sprot.fasta.gz [accessed 25 October 2018]) with DIAMOND v0.9.2 (24). BlobTools v1.1.1 (25) was used to find contigs that are likely of archaeal origin. MinION reads that mapped to these contigs were selected with SAMtools v1.7 (26). The selected MinION reads together with Illumina reads were assembled with SPAdes v3.11.0 (27, 28). The resulting scaffolds were binned with Centrifuge v1.0.4 (29). One scaffold was classified as archaeal by Centrifuge. This scaffold was polished twice with RACON v1.3.3 (30) using MinION reads and twice with Pilon v1.23 (31) using Illumina reads. NCBI BLAST v2.9.0+ was used to search for sequence similarity in the two ends of the linear genome. This resulted in an overlap of 56 bp. The overlapping 56 bp were removed from the end of the genome sequence. This metagenome assembly workflow resulted in a high-quality circular genome of 1.51 Mbp.

The circular genome enabled a thorough analysis of the genomic potential using Rapid Annotation using Subsystems Technology (RAST) (32, 33). The genome had a GC content of 43.7%, with 1,535 coding gene sequences, 44 tRNA genes, and 4 rRNA genes (one 16S rRNA gene, one 23S rRNA gene, and two 5S rRNA genes). The closest relative, based on the 16S rRNA gene sequence, was "*Candidatus* Methanogranum caenicola," with 97.7% sequence identity; the sequence identity to the 16S rRNA gene of *Methanomassiliicoccus luminyensis* 10B was 88.6%.

Data availability. The genome of *Methanomassiliicoccales* strain U3.2.1 is available in the NCBI database, with GenBank accession number CP076745.1. The whole-genome sequencing data are available under NCBI BioProject accession number PRJNA731838, with taxonomic identification number 1799672.

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