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Short Genome Communication

Genome sequence of *Methanobacterium congolense* strain Buetzberg, a hydrogenotrophic, methanogenic archaeon, isolated from a mesophilic industrial-scale biogas plant utilizing bio-waste

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ABSTRACT

Methanogenic *Archaea* are of importance at the end of the anaerobic digestion (AD) chain for biomass conversion. They finally produce methane, the end-product of AD. Among this group of microorganisms, members of the genus *Methanobacterium* are ubiquitously present in anaerobic habitats, such as bioreactors. The genome of a novel methanogenic archaeon, namely *Methanobacterium congolense* Buetzberg, originally isolated from a mesophilic biogas plant, was completely sequenced to analyze putative adaptive genome features conferring competitiveness of this isolate within the biogas reactor environment. Sequencing and assembly of the *M. congolense* Buetzberg genome yielded a chromosome with a size of 2,451,457 bp and a mean GC-content of 38.51%. Additionally, a plasmid with a size of 18,118 bp, featuring a GC content of 36.05% was identified. The *M. congolense* Buetzberg plasmid showed no sequence similarities with the plasmids described previously suggesting that it represents a new plasmid type. Analysis of the *M. congolense* Buetzberg chromosome architecture revealed a high collinearity with the *Methanobacterium paludis* chromosome. Furthermore, annotation of the genome and functional predictions disclosed several genes involved in cell wall and membrane biogenesis. Compilation of specific genes among *Methanobacterium* strains originating from AD environments revealed 474 genetic determinants that could be crucial for adaptation of these strains to specific conditions prevailing in AD habitats.

Methanogenic *Archaea* are ubiquitously present in anaerobic habitats including digestive tracts of animals, deep layers of marine sediments, hydrothermal vents, wetlands, rice fields, sewage sludge and biogas digestors (Angelidaki et al., 2011). Frequently, members of the mesophilic genus *Methanobacterium* belong to the archaeal sub-community within these habitats (Baserba et al., 2012; Xing et al., 2009). Currently, the genus *Methanobacterium* comprises 25 species originating from various environments as well as strains that have not been validly named yet, but nevertheless belong to the genus *Methanobacterium* based on their 16S rRNA gene sequence similarity to type strains of this genus (http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2160). All *Methanobacterium* species known so far are able to utilize carbon dioxide (CO_2) and hydrogen (H_2) as substrates for methanogenesis. Moreover, they feature a broad range of phenotypes and variations regarding their genomic specifications (Cadillo-Quiroz et al., 2014). *Methanobacterium* members are able to grow at pH values between 4.7 and 9.9 and temperatures ranging between 6 °C and 50 °C.

Besides the newly isolated strain *Methanobacterium congolense* from the biogas plant 'Buetzberg' near Hamburg (Germany), twelve further *Methanobacterium* genomes have been completely sequenced (Table 1). Nine of these methanogens were isolated from different natural environments; only two *Methanobacterium formicicum* strains were obtained from biogas reactors. For a twelfth isolate, namely the strain *Methanobacterium* sp. SMA-27, no information regarding its origin is publicly available (Table 1). Therefore, genomic data regarding *Methanobacterium* members or

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Table 1
Overview of sequenced *Methanobacterium* strains.

Strain	Source	Reference or accession number
M. arcticum M2^T	Permafrost environment	Shcherbakova et al. (2011)
M. sp. Mb1	Mesophilic biogas plants	Maus et al. (2013)
M. formicicum Mb9	Mesophilic biogas plants	ERS549551
M. formicicum MF^T	Sewage sludge	Maus et al. (2014)
M. formicicum BRM9	Cow rumen	Kelly et al. (2014)
M. formicicum DSM3637	Endosymbiont of *Pelomyxa palustris*	Gutierrez (2012)
M. lacus AL-21^T	Peatlands	Cadillo-Quiroz et al. (2014)
M. paludis SWAN-1^T	Peatlands	Cadillo-Quiroz et al. (2014)
M. sp. Maddingley MBC34	Coal-seam gas formation water metagenome	Rosewarne et al. (2013)
M. veterum MK4^T	Permafrost environment	Krivushin et al. (2010)
M. sp. SMA-27	No information available	NZ_JQLY00000000
M. sp. 42_16	Terrestrial metagenome	GCA_001509005.1
M. congolense Buetzberg	Mesophilic biogas plant Buetzberg	This work

Table 2
Genome features of *Methanobacterium congolense* strain Buetzberg.

Features	Chromosome	Plasmid
Size	2,451,457	18,118
GC content (%)	38.51	36.06
Protein coding genes	2319	24
rRNA operons	3	–
tRNA genes	43	–

other methanogens in mesophilic biogas plants presently are very rare.

Currently, the production of biogas from organic substrates is of increasing economic and energy political importance. Since *Methanobacterium* members are involved in bio-methanation from organic substrates (Boone and Mah, 2001), the question arises whether *Methanobacterium* strains from anaerobic digestion (AD) communities differ regarding their genomic features in comparison to *Methanobacterium* isolates originating from other environments. This study addresses the identification of genetic determinants potentially specifying competitiveness of *Methanobacterium* species in biogas reactor habitats. For this purpose, the genome sequence of *M. congolense* Buetzberg from the production-scale biogas plant Buetzberg (near Hamburg city, Germany) for bio-waste conversion (turnover 70,000 tons/year) was established and analyzed in detail. Moreover, strain Buetzberg is the first *M. congolense* isolate that has been sequenced so far (Table 2).

M. congolense Buetzberg was obtained from the mesophilic fermenter liquid (pH 7.6–8.0, 37 °C, high salt concentrations of on average 20–21 Siemens/cm, ammonium contents of around 2000 ppm and volatile fatty acids mostly below 500 ppm) of the biogas plant Buetzberg, utilizing bio-waste (separately collected garden green waste bins, vegetables as kitchen leftovers, no meat) as substrate for biomethanation. The bio-waste is put into batch garage-type fermenters without additional water and percolated by the squeezed liquids being collected in a separate liquid storage fermenter (De Baere and Mattheeuws, 2008; Siechau and Thoerner, 2012). The strain *M. congolense* Buetzberg was isolated as described previously applying the isolation method no. 11 (Maus et al., 2016). The 16S rRNA gene sequence analysis classified the strain Buetzberg as belonging to the species *M. congolense*, since its 16S rRNA gene sequence is 99% identical to the corresponding sequence of the reference strain *M. congolense* C^T (Cuzin et al., 2001). Moreover, the 23S rRNA and *mcrA* gene sequences of both strains also showed 99% similarity. The 16S rRNA gene sequence of *M. congolense* Buetzberg shares 99% identity with the non-type strain *Methanobacterium curvum* Px1 (Accession number AF276958). However, the species name *M. curvum* is not included in the List of Prokaryotic names with Standing in Nomenclature (LPSN, http://www.bacterio.net, accessed 12th of August 2016). For genome sequencing of *M. congolense* Buetzberg, its genomic DNA was extracted applying the GeneMATRIX Stool DNA Purification Kit (Roboklon, Germany). A sequencing library (with an average paired-end distance of 760 bp) was constructed and sequenced on the Illumina MiSeq system applying the paired-end protocol.

The sequencing approach resulted in 727,223 reads, accounting for 208,439,694 bases total sequence information. The Illumina reads were assembled by means of the GS *de novo* Assembler software (version 2.8, Roche) which resulted in 13 scaffolds comprising 27 contigs. Hence, the genome coverage approximately was 84-fold. Genome finishing was accomplished applying the CONSED software package (Gordon et al., 1998) and an *in silico* gap closure approach as described previously (Wibberg et al., 2011, 2014). The finished *M. congolense* Buetzberg chromosome has a size of 2,451,457 bases, featuring a GC-content of 38.51% (Fig. 1). An additional plasmid consisting of 18,118 bases with a GC content of 36.05% was identified (Fig. 1). Remarkably, the sequence read coverage observed for the plasmid was approximately twice as high as compared to the chromosome suggesting a higher plasmid copy number. Annotation of the genome within the GenDB 2.0 platform (Meyer et al., 2003) resulted in identification of 2319 protein-coding sequences, 43 tRNAs and three *rrn* operons. The plasmid comprises 24 protein-coding sequences; most of them were predicted to encode hypothetical proteins.

The bioinformatics programs r2cat (Husemann and Stoye, 2010) and MAUVE (Darling et al., 2004; Darling et al., 2010) were applied to compare the chromosomal architecture of *M. congolense* Buetzberg to those of other sequenced *Methanobacterium* strains. A high collinearity with the chromosome of *Methanobacterium paludis* SWAN-1 (Accession number CP002772) was observed (Fig. 2). A phylogenetic tree (Fig. 3) based on 1096 concatenated core genes shared by the completely sequenced *Methanobacterium* strains confirmed the close relationship between *M. congolense* Buetzberg and *M. paludis* SWAN-1. Average Nucleotide Identity (ANI) values were previously shown to be suitable for species demarcation (Richter and Rossello-Mora, 2009). An ANI analysis was performed as implemented in EDGAR 2.0 (Blom et al., 2016) and revealed a value of 84.62% between *M. congolense* Buetzberg and *M. paludis* SWAN-1 confirming that both strains really represent different species. ANI values calculated between *M. congolense* Buetzberg and the other sequenced *Methanobacterium* strains were even lower.

Additionally, phylogenetic analyses (Fig. 3) revealed the close relationship between the species *M. arcticum* M2 and *Methanobacterium veterum* MK4, casting doubt on affiliation of these strains to different species. The calculated ANI value for these strains was 99.99% and strongly suggests that both methanogens belong to the same species. Moreover, it appeared that *Methanobacterium* sp. 42_16, which has not yet been classified to any validly named species, may be assigned to the species *M. formicicum*. *Methanobac-*

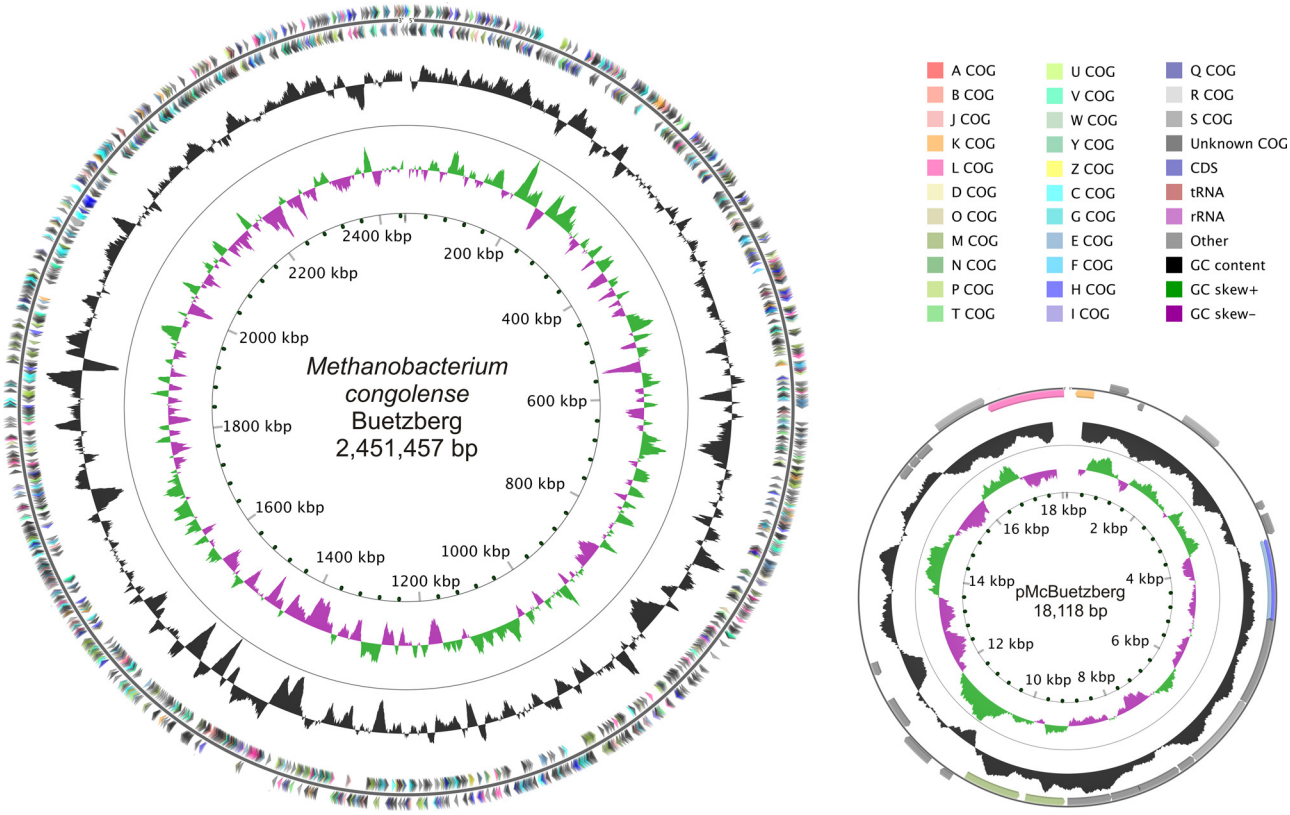

Fig. 1. Genome plots of the *M. congolense* Buetzberg chromosome and plasmid. From the inner to the outer circle: Circle 1: genomic position in kb; Circle 2: GC skew; Circle 3: GC content; Circles 3 and 4: predicted protein-coding sequences (CDS) transcribed clockwise (outer part) or anticlockwise (inner part). The CDSs are colored according to the assigned COG classes. The gene *cbiA* was chosen as the first gene of the chromosome. Plots were drawn with the CGView Server (Grant and Stothard, 2008).

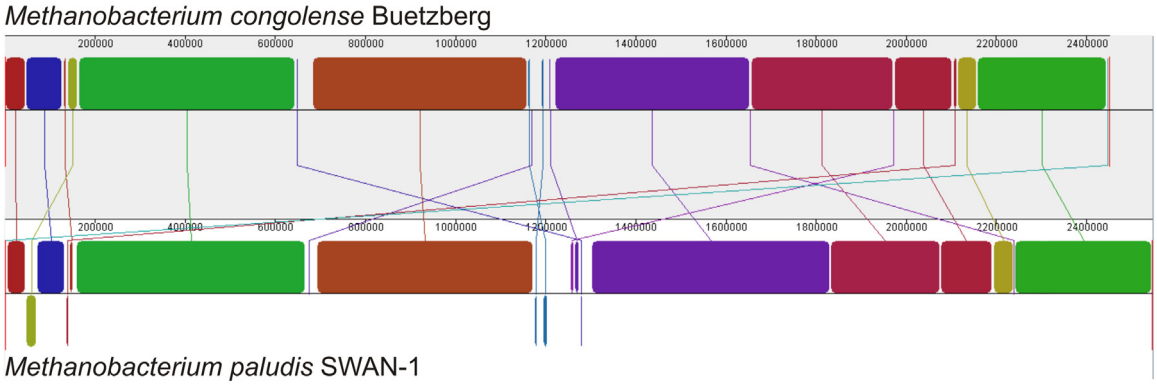

Fig. 2. Chromosomal architecture of *M. congolense* Buetzberg in comparison to *M. paludis* SWAN-1. A high collinearity was observed between the chromosomes of *M. congolense* Buetzberg and *M. paludis* SWAN-1. MAUVE plots were calculated by application of the progressive-Mauve algorithm implemented in the Mauve software 2.4.0 with default settings (Darling et al., 2004; Darling et al., 2010). A seed size of 15 was adjusted and the minimum Locally Collinear Blocks (LCBs) weight (3 times the minimum match size) was manually set to 3025 resulting in differentiation of 20 LCBs.

terium sp. 42_16 featured ANI values higher than 98% with different sequenced *M. formicicum* strains. Further analyses are required to confirm these findings. However, presented results may constitute cornerstones suggesting that corresponding strains should be reclassified.

As expected for a *Methanobacterium* strain, all genes required for hydrogenotrophic methanogenesis were identified in the *M. congolense* Buetzberg genome applying functional KEGG analyses (Kanehisa et al., 2016) implemented in GenDB. *M. congolense* Buetzberg possesses a central *ca.* 10 kb methanogenesis gene region (2,057,234–2,067,008 bp) encoding two enzymes, namely N5-methyl tetrahydromethanopterine: coenzyme M methyltransferase (Mtr) and methyl coenzyme M reductase (Mcr), which are involved in methane synthesis. Furthermore, the genome encodes the [NiFe] membrane-bound hydrogenases Eha and Ehb, described to exhibit different substrate specificities with respect to the electron acceptor and reduce different ferredoxins or poly-ferredoxins, which then might donate electrons to different oxidoreductases (Lie et al., 2012; Tersteegen and Hedderich, 1999).

Additional COG classification by means of the eggNOG database (Powell et al., 2012) revealed a similar distribution of COG categories as compared to *M. formicicum* BRM9 (Kelly et al., 2014). A total of 187 genes (~8%) were assigned to the category 'energy production and conversion' (functional COG category C) and 449

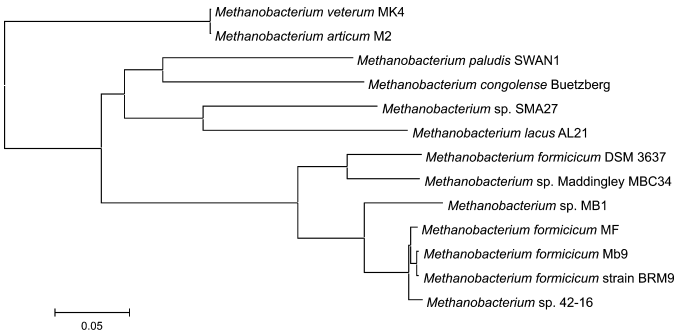

Fig. 3. Taxonomic classification of the isolate *M. congolense* Buetzberg. The phylogenetic analysis was done within the EDGAR 2.0 platform (Blom et al., 2016). In total, 1096 core genes were determined for the selected genomes, aligned using MUSCLE and concatenated to one large alignment. The Neighbor-Joining method in PHYLIP was used for the calculation of the phylogenetic tree. All nodes appeared to have bootstrap values of 100. The bar indicates 5 substitutions per 100 base pairs.

genes (~20%) are related to transport and metabolism of different molecules (COG categories E, F, G, H, I and P). However, around 64 genes (2.72%) involved in cell wall and membrane biogenesis (COG M) were identified in strain BRM9, whereas in *M. congolense* Buetzberg, 104 (4.49%) genetic determinants were assigned to the same functional category. This difference may reflect adaptations to specific environmental conditions.

To calculate the set of unique and common protein-coding genes of *Methanobacterium* strains obtained from anaerobic digestion (AD) habitats and isolates originating from other natural environments, the comparative genomics tool EDGAR 2.0 was applied. *Methanobacterium* sp. SMA-27 was not included in this analysis, since information on its origin is not available in the literature. This analysis revealed 1118 orthologous genes shared by twelve *Methanobacterium* genomes. These core genes were evaluated by KEGG annotation (Kanehisa et al., 2016). Among them are for example glycolysis pathway genes, genes for hydrogenotrophic methanogenesis and the conversion of formate to CO_2. In contrast, the group representing AD members revealed 474 unique genes with no orthologous counterparts in the genomes of the strains from other environments. Among these unique genes are 438 hypothetical genes (*ca.* 90% of the genes) and genes predicted to encode the K^+ ion uptake system Trk known from *Enterobacteriaceae* (Kempf and Bremer, 1998). In the latter study, it was shown that Trk is involved in K^+ ion uptake after osmotic shock in *Escherichia coli.* On the other hand, 2652 genes were only found in strains isolated from natural environments, and also 90% of them were predicted to encode hypothetical proteins. Further biochemical work is required to elucidate the role of these hypothetical genes in *Methanobacterium* species and whether they specify adaptive traits.

Analysis of the plasmid present in *M. congolense* Buetzberg revealed no sequence similarities with plasmids deposited in the NCBI database suggesting that it represents a new plasmid type. Out of 24 genes, seven are orthologous to corresponding genes identified in the *M. veterum* MK4 genome. These genes encode a phospho-adenosine phospho-sulfate reductase, a resolvase, the outer membrane assembly lipoprotein YfgL and 4 hypothetical proteins. Among the remaining 17 genes, 15 were annotated as hypothetical genes, while the two remaining genes encode a transcriptional regulator and a putative modification methylase. Plasmid replication genes could not be identified. None of the plasmid genes codes for known antibiotic resistance genes.

Availability of the complete genome sequence of the strain *M. congolense* Buetzberg now provides the opportunity to study differences in genome structures and contents among *Methanobacterium* species with the aim to identify potential adaptive features regarding their specific habitats and to exploit genome features to improve biomethanation.

Nucleotide sequence accession number

The *M. congolense* (strain Buetzberg) genome sequence was deposited in the European Nucleotide Archive (ENA) under the study accession number PRJEB14599 (http://www.ebi.ac.uk/ena/data/view/PRJEB14599) and the sequence Accession Numbers LT607756 (chromosome) and LT607757 (plasmid). The strain *M. congolense* Buetzberg is available from Prof. P. Scherer at the Laboratory for Applied Microbiology, Faculty Life Sciences, Research Center 'Biomass Utilization Hamburg', University of Applied Sciences Hamburg (HAW).

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