

GENOME ANNOUNCEMENT

## Complete Genome Sequence of the Hydrogenotrophic, Methanogenic Archaeon *Methanoculleus bourgensis* Strain MS2<sup>T</sup>, Isolated from a Sewage Sludge Digester

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*Methanoculleus bourgensis*, of the order *Methanomicrobiales*, is a dominant methanogenic archaeon in many biogas-producing reactor systems fed with renewable primary products. It is capable of synthesizing methane via the hydrogenotrophic pathway utilizing hydrogen and carbon dioxide or formate as the substrates. Here we report the complete and finished genome sequence of *M. bourgensis* strain MS2<sup>T</sup>, isolated from a sewage sludge digester.

he worldwide shortage and rising price of fossil energy, together with climate and environmental protection aims, have led to enhanced development and utilization of alternative energy resources. In this context, the production of biogas from microbial degradation of organic matter is of great importance, since biogas represents a clean and environmentally compatible energy source (7, 8). Methanogenic subcommunities within biogas-producing consortia are crucial in the anaerobic degradation process for synthesis of methane. Previous studies revealed that members of the genus Methanoculleus frequently dominate methanogenic subcommunities in different anaerobic digester systems (7, 9, 13, 19, 20). Methanoculleus marisnigri strain JR1, from a marine sediment, is the only member of the genus Methanoculleus that has been sequenced so far (1). Moreover, Methanoculleus bourgensis was identified in production-scale biogas plants fed with maize silage and manure (4, 7, 10). Other studies reported that M. bourgensis seems to be well adapted in biogas communities encountering high salt and ammonium concentrations (5, 16, 17).

*Methanoculleus bourgensis* strain MS2<sup>T</sup> was first isolated from a digester fermenting tannery by-products inoculated with digested sewage sludge from Bourg, France (12). This strain is a hydrogenotrophic methanogen utilizing hydrogen and carbon dioxide or formate as the substrates for methane synthesis. Acetate is required for growth (2). To analyze the adaptability of *M. bourgensis* MS2<sup>T</sup> to the conditions prevailing in biogas reactors, its genome was completely sequenced.

*M. bourgensis*  $MS2^T$  was purchased from the DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) and grown in modified DSMZ medium 287. Genomic DNA was isolated (14) and sequenced on the Genome Sequencer (GS) Junior system (Roche). A whole-genome shotgun and a paired-end sequencing run yielded 122,030 reads accounting for 57,786,764 bases of total sequence information. Thus, a 21-fold coverage was achieved for the 2.8-Mb *M. bourgensis*  $MS2^T$  genome. After assembling the sequence reads using the GS De Novo assembler software (version 2.6), the order of contigs was determined and gap closure was performed (18). The finished genome has a size of 2,789,773 bp, featuring a GC content of 60.64%. Annotation of the genome was accomplished within the GenDB platform (11), resulting in 2,619 coding sequences, with a mean gene length of 877 bp, 45 tRNA genes, and one *rrn* operon.

Comparative analysis (3, 6, 15) and interpretation of the genome sequence revealed that *M. bourgensis*  $MS2^{T}$  possesses a central 15,345-kb methanogenesis gene region encoding different enzymes involved in methane synthesis. Additional methanogenesis genes are scattered across the genome. Genes encoding highaffinity hydrogenases most likely involved in the utilization of hydrogen produced by syntrophic acetate or fatty acid-oxidizing microorganisms were identified. *M. bourgensis* MS2<sup>T</sup> possesses genes required for growth on acetate. Moreover, M. bourgensis MS2<sup>T</sup> has the genetic potential to produce osmolytes that could be crucial for the adaptation of the strain to the conditions prevailing in biogas reactors. The *M. bourgensis* MS2<sup>T</sup> genome will serve as a reference for comparative analyses comprising other methanogenic Archaea from biogas communities and guide studies on the adaptability of related organisms to the conditions in biogas reactor environments.

Nucleotide sequence accession number. The *M. bourgensis*  $MS2^{T}$  genome sequence was deposited at EBI under the accession number HE964772.

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## REFERENCES

- 1. Anderson IJ, et al. 2009. Complete genome sequence of *Methanoculleus* marisnigri Romesser et al. 1981. type strain JR1. Stand Genomic Sci. 1:189–196.
- Asakawa S, Nagaoka K. 2003. Methanoculleus bourgensis, Methanoculleus olentangyi and Methanoculleus oldenburgensis are subjective synonyms. Int. J. Syst. Evol. Microbiol. 53:1551–1552.
- 3. Eikmeyer F, et al. 2012. The complete genome sequences of four new IncN plasmids from wastewater treatment plant effluent provide new insights into IncN plasmid diversity and evolution. Plasmid 68:13–24.
- Feng XM, Karlsson A, Svensson BH, Bertilsson S. 2010. Impact of trace element addition on biogas production from food industrial wastelinking process to microbial communities. FEMS Microbiol. Ecol. 74: 226–240.
- 5. Goberna M, Insam H, Franke-Whittle IH. 2009. Effect of biowaste sludge maturation on the diversity of thermophilic bacteria and archaea in an anaerobic reactor. Appl. Environ. Microbiol. 75:2566–2572.
- Heinl S, et al. 20 March 2012. Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage. J. Biotechnol. [Epub ahead of print.] doi:10.1016/j.jbiotec.2012.03.007.
- Jaenicke S, et al. 2011. Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454pyrosequencing. PLoS One 6:e14519. doi:10.1371/journal.pone.0014519.
- 8. Jaenicke S, et al. 2011. Analysis of the metagenome from a biogas-producing microbial community by means of bioinformatics methods, p 403–414. *In* de Bruijn FJ (ed), Handbook of molecular microbial ecology II: metagenomics in different habitats. John Wiley & Sons, Inc., Hoboken, NJ.
- 9. Krause L, et al. 2008. Taxonomic composition and gene content of a methane-producing microbial community isolated from a biogas reactor. J. Biotechnol. 136:91–101.
- 10. Kröber M, et al. 2009. Phylogenetic characterization of a biogas plant microbial community integrating clone library 16S-rDNA sequences and

metagenome sequence data obtained by 454-pyrosequencing. J. Biotechnol. 142:38-49.

- 11. Meyer F, et al. 2003. GenDB—an open source genome annotation system for prokaryote genomes. Nucleic Acids Res. 31:2187–2195.
- Ollivier BM, Mah RA, Garcia JL, Boone DR. 1986. Isolation and characterization of *Methanogenium bourgense*. sp. nov. Int. J. Syst. Bacteriol. 36:297–301.
- 13. Rademacher A, et al. 2012. Characterization of microbial biofilms in a thermophilic biogas system by high-throughput metagenome sequencing. FEMS Microbiol. Ecol. **79**:785–799.
- Schlüter A, et al. 2008. The metagenome of a biogas-producing microbial community of a production-scale biogas plant fermenter analysed by the 454-pyrosequencing technology. J. Biotechnol. 136:77–90.
- Schneiker-Bekel S, et al. 2011. The complete genome sequence of the dominant *Sinorhizobium meliloti* field isolate SM11 extends the *S. meliloti* pan-genome. J. Biotechnol. 155:20–33.
- Schnürer A, Zellner G, Svensson BH. 1999. Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. FEMS Microbiol. Ecol. 29:249–261.
- Weiss A, et al. 2009. Investigation of factors influencing biogas production in a large-scale thermophilic municipal biogas plant. Appl. Microbiol. Biotechnol. 84:987–1001.
- Wibberg D, et al. 2011. Complete genome sequencing of Agrobacterium sp. H13-3, the former *Rhizobium lupini* H13-3, reveals a tripartite genome consisting of a circular and a linear chromosome and an accessory plasmid but lacking a tumor-inducing Ti-plasmid. J. Biotechnol. 155:50–62.
- Wirth R, et al. 2012. Characterization of a biogas-producing microbial community by short-read next generation DNA sequencing. Biotechnol. Biofuels 5:41.
- 20. Zakrzewski M, et al. 2012. Profiling of the metabolically active community from a production-scale biogas plant by means of high-throughput metatranscriptome sequencing. J. Biotechnol. 158:248–258.