AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements

Complete Genome Sequence of the Methanogen *Methanoculleus bourgensis* BA1 Isolated from a Biogas Reactor

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***Methanoculleus bourgensis* BA1, a hydrogenotrophic methanogen, was isolated from a laboratory-scale biogas reactor operating under an elevated ammonium concentration. Here, the complete genome sequence of *M. bourgensis* BA1 is reported. The availability of the BA1 genome sequence enables detailed comparative analyses involving other *Methanoculleus* spp. representing important members of microbial biogas communities.**

Received 4 May 2016 **Accepted** 10 May 2016 **Published** 23 June 2016

Citation Maus I, Wibberg D, Winkler A, Pühler A, Schnürer A, Schlüter A. 2016. Complete genome sequence of the methanogen *Methanoculleus bourgensis* BA1 isolated from a biogas reactor. Genome Announc 4(3):e00568-16. doi:10.1128/genomeA.00568-16.

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Frequently, members of the genus *Methanoculleus* were described as playing an important role in different biogas reactor systems (1, 2). In particular, the species *Methanoculleus bourgensis* was found to be dominant in several biogas systems. Moreover, different studies described the prevalence of *M. bourgensis* in reactors performing syntrophic acetate oxidation (SAO) under high ammonium concentrations (3–5), indicating the importance of this methanogen in corresponding communities. Isolation and/or cocultivation of *M. bourgensis*, together with acetate-oxidizing bacteria (4, 6) such as *Clostridium ultunense* (7), led to the assumption that syntrophic association may play an important role for members of the genus *Methanoculleus*. Bioaugmentation involving *Methanoculleus* spp. in coculture with SAO bacteria was discussed as a feasible approach to shorten the adaptation period of digesters operating under high ammonium/ammonia concentrations (3, 8).

The objective of this work was to sequence the methanogen *M. bourgensis* BA1 (9) originating from a Swedish lab-scale continuous stirred tank reactor (37°C) operating under an elevated ammonium concentration (6.4 g l^{-1} NH_4+ N) and utilizing alfalfa silage for methane production. Furthermore, the availability of the *M. bourgensis* BA1 genome sequence and insights into its predicted metabolic capabilities provide reference points for comparative analyses comprising other methanogenic species of *Archaea* from biogas communities.

Strain BA1 was isolated as described previously (9, 10). The 16S rRNA gene sequence analysis classified the isolate as a member of the species *M. bourgensis* with 99% sequence identity to the 16S rRNA gene of strain MS2^T (11). Genomic DNA of strain BA1 was isolated using the Qiagen blood and tissue kit and sequenced applying the paired-end protocol on an Illumina MiSeq system. The 2,155,212 reads obtained, accounting for 565,780,211 bp of sequence information, were *de novo* assembled using the GS *de novo* assembler version 2.8 software. The assembly resulted in 14 scaffolds comprising 48 contigs. An *in silico* gap closure approach (12) was applied to close all gaps between contigs and circularize the genome. The complete BA1 chromosome has a size of 2,551,189 bp, featuring a GC content of 60.89%. Annotation of the genome sequence was performed within the annotation system GenDB version 2.0 (13) and resulted in the detection of 2,528 protein-coding sequences, 45 tRNA genes, and one *rrn* operon.

Interpretation of the *M. bourgensis* BA1 genome sequence revealed that all genes required for hydrogenotrophic methanogenesis were identified. Moreover, genes encoding a formate transporter (*fdhC*) and a formate dehydrogenase operon (*fdhA-B*) for growth on formate as an alternative methanogenic substrate were found. Since strain BA1 was isolated from a habitat rich in ammonium/ammonia, genes involved in nitrogen metabolism were analyzed. Similar to the type strain *M. bourgensis* MS2^T (11, 14), the BA1 genome encodes neither a methylammonium permease nor the putative archaeal ammonium uptake system Amt predicted to transport NH_4+. The missing ammonium transporter may indicate an adaptation of the strain to environments rich in ammonium/ammonia. Furthermore, strain BA1 harbors genes encoding different potassium transporters and a glycine betaine/proline transport system that may contribute to compatible solute accumulation as response to high osmolarity.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited in the EMBL/GenBank database (EBI, NCBI) under the accession number LT549891 (Study ID: PRJEB13327).

ACKNOWLEDGMENTS

The bioinformatics support of the BMBF-funded project "Bielefeld-Gießen Center for Microbial Bioinformatics"—BiGi (grant 031A533) within the German Network for Bioinformatics Infrastructure (de.NBI) is gratefully acknowledged. I.M. and D.W. acknowledge the receipt of a scholarship from the CLIB Graduate Cluster "Industrial Biotechnology," cofinanced by the Ministry of Innovation of North Rhine-Westphalia. Moreover, we acknowledge funding by the German Federal Ministry of

Food and Agriculture (BMEL, grant 22006712), and the German Federal Ministry of Education and Research (BMBF, grant 03SF0440C).

FUNDING INFORMATION

This work, including the efforts of Alfred Pühler, was funded by German Federal Ministry of Education and Research (031A533 and 03SF0440C). This work, including the efforts of Alfred Pühler, was funded by German Federal Ministry of Food and Agriculture (22006712 and 22404015).

REFERENCES

1. **Jaenicke S, Ander C, Bekel T, Bisdorf R, Dröge M, Gartemann KH, Jünemann S, Kaiser O, Krause L, Tille F, Zakrzewski M, Pühler A, Schlüter A, Goesmann A.** 2011. Comparative and joint analysis of two metagenomic datasets from a biogas fermenter obtained by 454-pyrosequencing. PLoS One **6:**e14519. http://dx.doi.org/10.1371/journal.pone.0014519.
2. **Stolze Y, Zakrzewski M, Maus I, Eikmeyer F, Jaenicke S, Rottmann N, Siebner C, Pühler A, Schlüter A.** 2015. Comparative metagenomics of biogas-producing microbial communities from production-scale biogas plants operating under wet or dry fermentation conditions. Biotechnol Biofuels **8:**14. http://dx.doi.org/10.1186/s13068-014-0193-8.
3. **Westerholm M, Levén L, Schnürer A.** 2012. Bioaugmentation of syntrophic acetate-oxidizing culture in biogas reactors exposed to increasing levels of ammonia. Appl Environ Microbiol **78:**7619–7625. http://dx.doi.org/10.1128/AEM.01637-12.
4. **Moestedt J, Müller B, Westerholm M, Schnürer A.** 2016. Ammonia threshold for inhibition of anaerobic digestion of thin stillage and the importance of organic loading rate. Microb Biotechnol **9:**180–194. http://dx.doi.org/10.1111/1751-7915.12330.
5. **Westerholm M, Müller B, Isaksson S, Schnürer A.** 2015. Trace element and temperature effects on microbial communities and links to biogas digester performance at high ammonia levels. Biotechnol Biofuels **8:**154. http://dx.doi.org/10.1186/s13068-015-0328-6.
6. **Fotidis IA, Karakashev D, Angelidaki I.** 2013. Bioaugmentation with an acetate-oxidising consortium as a tool to tackle ammonia inhibition of anaerobic digestion. Bioresour Technol **146:**57–62. http://dx.doi.org/10.1016/j.biortech.2013.07.041.
7. **Schnürer A, Schink BH, Svensson BH.** 1996. *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. Int J Syst Bacteriol **46:**1145–1152. http://dx.doi.org/10.1099/00207713-46-4-1145.
8. **Fotidis IA, Wang H, Fiedel NR, Luo G, Karakashev DB, Angelidaki I.** 2014. Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate. Environ Sci Technol **48:**7669–7676. http://dx.doi.org/10.1021/es5017075.
9. **Schnürer A, Zellner G, Svensson BH.** 1999. Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. FEMS Microbiol Ecol **29:**249–261.
10. **Zehnder AJ, Huser BA, Brock TD, Wuhrmann K.** 1980. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch Microbiol **124:**1–11. http://dx.doi.org/10.1007/BF00407022.
11. **Maus I, Wibberg D, Stantscheff R, Stolze Y, Blom J, Eikmeyer FG, Fracowiak J, König H, Pühler A, Schlüter A.** 2014. Insights into the annotated genome sequence of *Methanoculleus bourgensis* MS2(T), related to dominant methanogens in biogas-producing plants. J Biotechnol **201:**43–53. http://dx.doi.org/10.1016/j.jbiotec.2014.11.020.
12. **Wibberg D, Blom J, Jaenicke S, Kollin F, Rupp O, Scharf B, Schneiker-Bekel S, Sczcepanowski R, Goesmann A, Setubal JC, Schmitt R, Pühler A, Schlüter A.** 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. http://dx.doi.org/10.1016/j.jbiotec.2011.01.010.
13. **Meyer F, Goesmann A, McHardy AC, Bartels D, Bekel T, Clausen J, Kalinowski J, Linke B, Rupp O, Giegerich R, Pühler A.** 2003. GenDB—an open source genome annotation system for prokaryote genomes. Nucleic Acids Res **31:**2187–2195. http://dx.doi.org/10.1093/nar/gkg312.
14. **Maus I, Wibberg D, Stantscheff R, Eikmeyer FG, Seffner A, Boelter J, Szczepanowski R, Blom J, Jaenicke S, König H, Pühler A, Schlüter A.** 2012. Complete genome sequence of the hydrogenotrophic, methanogenic archaeon *Methanoculleus bourgensis* strain MS2(T), isolated from a sewage sludge digester. J Bacteriol **194:**5487–5488. http://dx.doi.org/10.1128/JB.01292-12.