

Complete Genome Sequence of *Methanoregula formicica* SMSPT^T, a Mesophilic Hydrogenotrophic Methanogen Isolated from a Methanogenic Upflow Anaerobic Sludge Blanket Reactor

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***Methanoregula formicica* SMSPT^T is a mesophilic H₂/formate-utilizing methanogenic archaeon and a representative of the family *Methanoregulaceae*, a recently proposed novel family within the order *Methanomicrobiales*. Here, we report a 2.8-Mb complete genome sequence of this methanogenic archaeon.**

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Methanoregula formicica SMSPT^T, a mesophilic H₂/formate-utilizing methanogen, was isolated from methanogenic granular sludge in an upflow anaerobic sludge blanket (UASB) reactor in Japan and described as a novel species within the order *Methanomicrobiales* (1). *M. formicica* SMSPT^T belongs to the family *Methanoregulaceae*, a recently proposed novel family within the order *Methanomicrobiales* (2). The family *Methanoregulaceae* comprises five valid species, *M. formicica* SMSPT^T, *Methanoregula boonei* 6A8^T (3), *Methanolinea tarda* NOBI-1^T (4), *Methanolinea mesophila* TNR^T (2), and *Methanosphaerula palustris* E1-9c^T (5). Although these strains were taxonomically identified mainly by molecular phylogeny and classified into the single family *Methanoregulaceae*, common genomic features shared by *Methanoregulaceae* species are still largely unclear. Here we report the complete genome sequence of *M. formicica* SMSPT^T, which provides insight into the unique physiological and genetic features of the species within the family *Methanoregulaceae*.

The whole-genome shotgun sequencing was performed using a combined Roche GS-FLX Titanium and Illumina GAii approach. Sequence assembly was carried out using ALLPATHS (version R41043) (6), Velvet (version 1.1.05) (7), and Phrap (version SPS 4.24; High Performance Software, LLC). Manual finishing efforts raised the quality of the assembly to that of a finished genome. Genes were identified using Prodigal (8) as part of the JGI genome annotation pipeline (9), followed by a round of manual curation using the JGI GenePRIMP pipeline (10). Additional gene functional annotation and comparative analysis were performed within the Integrated Microbial Genomes (IMG-ER) platform (11).

The complete genome is 2,820,858 bp with a G + C content of 55.2%. The genome contains 2,870 protein-coding sequences, 54

pseudo genes, 49 tRNA genes, and an rRNA operon including 5S, 16S, and 23S subunit genes. A total of 69.3% of open reading frames (2,027) are protein-coding genes with function prediction.

Gene classification by the NCBI clusters of orthologous groups (COG) categories (12) reveals that major cellular processes are energy production and conversion, translation and transcription, signal transduction, transport and metabolism of amino acids/coenzymes/inorganic ions. The genome harbors the genes encoding formate dehydrogenase, which is essential for formate utilization for growth and methane production. This underpins the formate-dependent growth of *M. formicica* SMSPT^T. The genome possesses the complete gene set for the acetyl-CoA decarbonylase/synthase (ACDS) multienzyme complex, which catalyzes reversible reactions, i.e., the reversible cleavage and synthesis of acetyl-CoA. Although acetate does not support the growth and/or methane production of *M. formicica* SMSPT^T (1), ACDS can be used for anabolic carbon dioxide fixation. The genetic, metabolic, and physiological features of the species belonging to the family *Methanoregulaceae* will be unveiled by comparative genomic analyses with other *Methanoregulaceae* species and/or methanogens within other taxa.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in DDBJ/EMBL/GenBank under the accession number CP003167. The version described in this paper is the first version, CP003167.1.

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REFERENCES

1. Yashiro Y, Sakai S, Ehara M, Miyazaki M, Yamaguchi T, Imachi H. 2011. *Methanoregula formicica* sp. nov., a methane-producing archaeon isolated from methanogenic sludge. *Int. J. Syst. Evol. Microbiol.* 61:53–59. <http://dx.doi.org/10.1099/ijs.0.014811-0>.
2. Sakai S, Ehara M, Tseng I-C, Yamaguchi T, Bräuer SL, Cadillo-Quiroz H, Zinder SH, Imachi H. 2012. *Methanolinea mesophila* sp. nov., a hydrogenotrophic methanogen isolated from rice field soil, and proposal of the archaeal family *Methanoregulaceae* fam. nov. within the order *Methanomicrobiales*. *Int. J. Syst. Evol. Microbiol.* 62:1389–1395. <http://dx.doi.org/10.1099/ijs.0.035048-0>.
3. Bräuer SL, Cadillo-Quiroz H, Ward RJ, Yavitt JB, Zinder SH. 2011. *Methanoregula boonei* gen. nov., sp. nov., an acidophilic methanogen isolated from an acidic peat bog. *Int. J. Syst. Evol. Microbiol.* 61:45–52. <http://dx.doi.org/10.1099/ijs.0.021782-0>.
4. Imachi H, Sakai S, Sekiguchi Y, Hanada S, Kamagata Y, Ohashi A, Harada H. 2008. *Methanolinea tarda* gen. nov., sp. nov., a methane-producing archaeon isolated from a methanogenic digester sludge. *Int. J. Syst. Evol. Microbiol.* 58:294–301. <http://dx.doi.org/10.1099/ijs.0.65394-0>.
5. Cadillo-Quiroz H, Yavitt JB, Zinder SH. 2009. *Methanosphaerula palustris* gen. nov., sp. nov., a hydrogenotrophic methanogen isolated from a minerotrophic fen peatland. *Int. J. Syst. Evol. Microbiol.* 59:928–935. <http://dx.doi.org/10.1099/ijs.0.006890-0>.
6. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. *Proc. Natl. Acad. Sci. U. S. A.* 108:1513–1518. <http://dx.doi.org/10.1073/pnas.1017351108>.
7. Zerbino DR, Birney E. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829. <http://dx.doi.org/10.1101/gr.074492.107>.
8. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <http://dx.doi.org/10.1186/1471-2105-11-119>.
9. Mavromatis K, Ivanova NN, Chen IM, Szeto E, Markowitz VM, Kyrpides NC. 2009. The DOE-JGI standard operating procedure for the annotations of microbial genomes. *Stand. Genomic Sci.* 1:63–67. <http://dx.doi.org/10.4056/sigs.632>.
10. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. 2010. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. *Nat. Methods* 7:455–457. <http://dx.doi.org/10.1038/nmeth.1457>.
11. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. 2009. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* 25:2271–2278. <http://dx.doi.org/10.1093/bioinformatics/btp393>.
12. Tatusov RL, Natale DA, Garkavtsev IV, Tatusova TA, Shankavaram UT, Rao BS, Kiryutin B, Galperin MY, Fedorova ND, Koonin EV. 2001. The COG database: new developments in phylogenetic classification of proteins from complete genomes. *Nucleic Acids Res.* 29:22–28. <http://dx.doi.org/10.1093/nar/29.1.22>.