

# Complete genome sequence of *Methylococcus geothermalis* IM1<sup>T</sup> isolated from a geothermal field

Samuel Imisi Awala , Joo-Han Gwak , Yong-Man Kim, Chanmee Seo , and Sung-Keun Rhee\* 

Department of Biological Sciences and Biotechnology, Chungbuk National University, Cheongju 28644, Republic of Korea

## 온천으로부터 분리된 *Methylococcus geothermalis* IM1<sup>T</sup> 균주의 유전체 염기서열

사무엘 이미시 아왈라  · 광주한  · 김용만 · 서찬미  · 이성근\* 

충북대학교 생명시스템학과

(Received April 27, 2022; Revised May 20, 2022; Accepted May 21, 2022)

The genome of *Methylococcus geothermalis* strain IM1<sup>T</sup>, a moderately thermophilic aerobic methanotroph isolated from a geothermal field in South Korea, was sequenced. This bacterium possesses genes encoding the particulate and soluble membrane monooxygenase necessary for methane oxidation, and enzymes required for the following reaction steps converting methanol to CO<sub>2</sub>. Genes for carbon assimilation pathways including the ribulose monophosphate, serine, and Calvin-Benson-Bassham cycles, assimilation of various nitrogen compounds, and detoxification of reactive nitrogen species such as hydroxylamine oxidoreductase were also found.

**Keywords:** *Methylococcus*, genome, methane, methanotroph

Members of the genus *Methylococcus* are thermotolerant or moderately thermophilic methanotrophs utilizing methane as the sole carbon and energy source (Bowman, 2015). They possess both particulate methane monooxygenase (pMMO) and soluble methane monooxygenase (sMMO) (Bowman, 2015). They are typically found in environments such as sewage, freshwaters, geothermal fields, and landfills (Malashenko *et al.*, 1975; Bowman *et al.*, 1993). Members of this genus have numerous biotechnological applications, including bioremediation

of pollutants and conversion of methane into commercially valuable products (Semrau, 2011; Semrau and DiSpirito, 2019). Here, we report the complete genome sequence of a recently described member of this genus, *Methylococcus geothermalis* IM1<sup>T</sup> (Awala *et al.*, 2020).

Strain IM1<sup>T</sup> was grown in a low salt mineral (LSM) medium with methane as the sole carbon and energy source under constant agitation (200 rpm) at 42°C. High molecular-weight genomic DNA was extracted from this culture using a previously described modified CTAB method (Hurt *et al.*, 2001). Whole-genome sequencing was performed using the PacBio RS II and Illumina HiSeq (2 × 150 bp) platforms at Macrogen. Long-read sequencing from the PacBio RS II platform was used for *de novo* assembly with HGAP assembler (v3.0) (Chin *et al.*, 2013). Error correction of the assembled genome was performed with Pilon (v1.23; default settings with three iterations) (Walker *et al.*, 2014) using the HiSeq reads. Annotation of the assembled genome was performed using the NCBI Prokaryotic Genome Annotation Pipeline v5.3 (Tatusova *et al.*, 2016).

Strain IM1<sup>T</sup> has a circular chromosome of 3,371,031 bp in size and a G + C content of 63.25%. Other genomic features are presented in Table 1. Genes required for methanotrophic traits were found in strain IM1<sup>T</sup> genome. These include two nearly identical copies of the *pmoCAB* operon and a single *pmoC*

\*For correspondence. E-mail: rhees@chungbuk.ac.kr;  
Tel.: +82-43-261-2300; Fax: +82-43-264-9600

**Table 1. Genomic features of *M. geothermalis* IM1<sup>T</sup>**

Feature type	Value
Size (bp)	3,371,031
Contigs	1
Circular chromosome	Yes
G + C content (%)	63.25
Contig N50	3,371,031
Genes (total)	3,147
CDS (total)	3,090
CDS (with protein)	2,985
rRNAs (5S, 16S, 23S)	2, 2, 2
tRNAs	47
ncRNAs	3
GenBank Accession	CP046565

paralog encoding the pMMO. A gene cluster (*mmoRSQG*-hypothetical protein-*mmoCDZBYX*) containing components of the sMMO was also detected. Methanol from methane oxidation is oxidized to formaldehyde using the pyrroloquinoline quinone (PQQ)-dependent methanol dehydrogenases. Gene clusters for PQQ biosynthesis (*pqqABCDE*), calcium- (*mxoEYFJGIRSACKLD*), and lanthanide- (*xoxFJ* and *xoxG*) dependent methanol dehydrogenases were found.

Genes for formaldehyde oxidation to CO<sub>2</sub> via formate, and biomass generation via the serine, ribulose monophosphate (RuMP), and Calvin-Benson-Bassham (CBB) cycles were found. These include (1) genes for the tetrahydromethanopterin- (*fae*, *fhcCDAB*, *mch*, *mptG*, *mtdB*) and tetrahydrofolate- (*fch*, *fhs*, and *mtDA*) dependent formaldehyde oxidation, (2) genes encoding different orthologs of formate dehydrogenase, and (3) key genes of the serine (*sgaA*, *hpr*, and *gck*), RuMP (*phi* and *hps*), and CBB (*cbbSL*) cycles.

Nitrogen-metabolism genes were also present in the genome. A gene cluster containing an assimilatory nitrate reductase (*nasA*), nitrite reductase (*nirBD*), and nitrate transporter was present. Strain IM1<sup>T</sup> also possessed genes encoding hydroxylamine oxidoreductase (*haoAB*), nitric oxide reductase (*norBC*), and nitrogenase (*nifHDK* and *nifENX*).

#### Nucleotide sequence accession number

The whole-genome sequence accession number for *Methylococcus geothermalis* IM1<sup>T</sup> in GenBank is CP046565. The strain accession numbers at the Korean Collection for Type

Cultures and Japan Collection of Microorganisms are KCTC 72677 and JCM 33941, respectively.

## 적 요

한국의 온천에서 분리한 *Methylococcus* 속의 고온성 호기성 메탄산화균의 유전체를 분석하였다. 이 세균은 메탄의 산화에 관여하는 핵심 유전자(pMMO 와 sMMO)를 포함하여 전체 메탄 산화 과정에 관여하는 다양한 유전자를 보유하고 있다. 또한, 탄소 고정을 위한 대사(ribulose monophosphate, serine, 그리고 Calvin-Benson-Bassham cycle)를 포함하여 다양한 질소 대사 과정(질산 및 질소 동화; hydroxylamine 무독화)에 관여하는 유전자를 보유하고 있다.

## Acknowledgments

This work was financially supported by the Research Year of Chungbuk National University in 2019 and the National Institute of Agricultural Science, Ministry of Rural Development Administration, Republic of Korea (research project PJ01700703).

## Conflict of Interest

The authors declare no notable conflict of interest.

## References

- Awala SI, Bellosillo LA, Gwak JH, Nguyen NL, Kim SJ, Lee BH, and Rhee SK.** 2020. *Methylococcus geothermalis* sp. nov., a methanotroph isolated from a geothermal field in the Republic of Korea. *Int. J. Syst. Evol. Microbiol.* **70**, 5520–5530.
- Bowman JP.** 2015. *Methylococcus*. In Trujillo ME, Dedysh S, DeVos P, Hedlund B, Kämpfer P, Rainey FA, and Whitman WB. (eds.), *Bergey's Manual of Systematics of Archaea and Bacteria*. John Wiley & Sons, Inc, Hoboken, New Jersey, USA.
- Bowman JP, Sly LI, Nichols PD, and Hayward AC.** 1993. Revised taxonomy of the methanotrophs: description of *Methylobacter* gen. nov., emendation of *Methylococcus*, validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family *Methylococcaceae* includes only the group I methanotrophs. *Int. J. Syst. Evol. Microbiol.* **43**, 735–753.
- Chin CS, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C,**

- Clum A, Copeland A, Huddleston J, Eichler EE, *et al.*** 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat. Methods* **10**, 563–569.
- Hurt RA, Qiu X, Wu L, Roh Y, Palumbo A, Tiedje JM, and Zhou J.** 2001. Simultaneous recovery of RNA and DNA from soils and sediments. *Appl. Environ. Microbiol.* **67**, 4495–4503.
- Malashenko IP, Romanovskaia VA, Bogachenko VN, and Shved AD.** 1975. Thermophilic and thermotolerant bacteria that assimilate methane. *Mikrobiologiya* **44**, 855–862.
- Semrau JD.** 2011. Bioremediation via methanotrophy: overview of recent findings and suggestions for future research. *Front. Microbiol.* **2**, 209.
- Semrau JD and DiSpirito AA.** 2019. Methanotrophy - environmental, industrial and medical applications. *Curr. Issues Mol. Biol.* **33**, 1–22.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, and Ostell J.** 2016. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res.* **44**, 6614–6624.
- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, *et al.*** 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS ONE* **9**, e112963.