

# Genome Sequence of the Obligate Gammaproteobacterial Methanotroph *Methylobacterium album* Strain BG8

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**The complete genome sequence of *Methylobacterium album* strain BG8, a methane-oxidizing gammaproteobacterium isolated from freshwater, is reported. Aside from a conserved inventory of genes for growth on single-carbon compounds, *M. album* BG8 carries a range of gene inventories for additional carbon and nitrogen transformations but no genes for growth on multicarbon substrates or for N fixation.**

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Methanotrophic bacteria are found in diverse environments and utilize methane as their sole source of energy, reductants, and carbon (1). Methanotrophs attenuate the emission of methane, the second most important greenhouse gas (2), and have applications in bioremediation and bioprocessing (3). *Methylobacterium album* strain BG8 (also known as *Methylobacter albus*, *Methylomonas albus*, and *Methylomonas alba*) is a mesophilic, aerobic gammaproteobacterium isolated from freshwater by Roger Whittenbury et al. (4).

The *M. album* BG8 genome was sequenced, assembled, and annotated by the U.S. Department of Energy Joint Genome Institute (<http://www.jgi.doe.gov/sequencing/>). Illumina GA II and 454 Titanium standard libraries with paired-end reads were generated, representing 30-fold coverage. Using Newbler v2.3, a chromosomal sequence of 2 contigs and 1 scaffold and a complete plasmid sequence were assembled. Automatic annotation was performed using Prodigal and GenePRIMP (5). The draft genome is 4.49 Mbp, with a mean G+C content of 56.2%. Two copies of the rRNA operon, 42 tRNA genes, and 3,984 predicted protein-coding genes are present. Manual annotation and comparative analysis are under way with assistance from the MicroScope annotation platform at Genoscope (6).

The *M. album* BG8 genome contains one operon (*pmoCAB*) with genes encoding particulate methane monooxygenase and a *pxm* operon (*pxmABC*) with genes encoding a copper membrane monooxygenase of unknown function (7). Genes encoding the enzyme methanol dehydrogenase and accessory proteins (*mxoYDFGIRSACKL-mxaB*) and a Xox-type methanol dehydrogenase (*xoxFJ*) (8) were identified. Genes encoding enzymes for C<sub>1</sub> metabolism include those for the tetrahydrofolate (H<sub>4</sub>-folate) and

tetrahydromethanopterin (H<sub>4</sub>MPT)-linked C<sub>1</sub> transfer pathways, glutathione-dependent formaldehyde dehydrogenases (GD-FALDH) and accessory functions, and a NAD-dependent formate dehydrogenase (encoded by *fdh5A*). A membrane-bound formate dehydrogenase (encoded by *fdh3DABC*) is typically absent in strict methanotrophs, but it is expressed in “*Candidatus* Methylophilum fumarolicum” SolV of the phylum *Verrucomicrobia*. Also similar to “*Ca. Methylophilum fumarolicum*” SolV, *M. album* BG8 was found to have a complete set of genes for glycogen biosynthesis (9). Genes encoding the complete ribulose monophosphate (RuMP) pathway for formaldehyde assimilation, a complete tricarboxylic acid (TCA) cycle, the pentose phosphate pathway, and the Embden-Meyerhof-Parnas pathway were identified. A complement of genes for a complete serine cycle, with the exception of phosphoenolpyruvate carboxylase, was identified; key genes for the Calvin-Benson-Bassham cycle were absent.

Genes encoding enzymes for nitrogen metabolism were identified, including those for direct ammonium uptake (*amtB*), nitrate transport (*narK*), nitrate or nitrite reduction (*nasCA*, *nasB*, and *nirBD*), and glutamine synthetase/glutamate synthase (*glnA*, *gltB*) and alanine dehydrogenase (*ald*) for ammonium assimilation. Tandem genes encoding proteins implicated in the oxidation of hydroxylamine to nitrite (*haoAB*) were identified, which likely facilitate the detoxification of hydroxylamine produced from the oxidation of ammonia by membrane-bound methane monooxygenase (pMMO) (10). Genes encoding a cytochrome *cd*<sub>1</sub> nitrite reductase (*nirS*) and accessory functions, as well as nitric oxide reductase (*norCB*), were found, perhaps explaining the capacity of this strain to tolerate high nitrite concentrations (11). The *nirS* and *norCB* genes in this strain share high sequence identities with

homologues in other methanotrophs (12, 13). Analysis of the *M. album* BG8 genome sequence enables further understanding of single-carbon metabolism and the environmental adaptation strategies of methanotrophs.

**Nucleotide sequence accession numbers.** The genome sequences of the chromosome and plasmid of *M. album* BG8 have been deposited in GenBank under accession no. [CM001475](#) and [CM001476](#), respectively.

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## REFERENCES

1. Trotsenko YA, Murrell JC. 2008. Metabolic aspects of aerobic obligate methanotrophy. *Adv. Appl. Microbiol.* **63**:183–229.
2. Conrad R. 2009. The global methane cycle: recent advances in understanding the microbial processes involved. *Environ. Microbiol. Rep.* **1**:285–292.
3. Han JI, Lontoh S, Semrau JD. 1999. Degradation of chlorinated and brominated hydrocarbons by *Methylobacterium album* BG8. *Arch. Microbiol.* **172**:393–400.
4. Whittenbury R, Phillips KC, Wilkinson JF. 1970. Enrichment, isolation and some properties of methane-utilizing bacteria. *J. Gen. Microbiol.* **61**: 205–218.
5. 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.
6. Vallenet D, Engelen S, Mornico D, Cruveiller S, Fleury L, Lajus A, Rouy Z, Roche D, Salvignol G, Scarpelli C, Médigue C. 2009. MicroScope: a platform for microbial genome annotation and comparative genomics. Database 2009:bap021. doi:<http://dx.doi.org/10.1093/database/bap021>.
7. Tavormina PL, Orphan VJ, Kalyuzhnaya MG, Jetten M, Klotz MG. 2011. A novel family of functional operons encoding methane/ammonia monooxygenase-related proteins in gammaproteobacterial methanotrophs. *Environ. Microbiol. Rep.* **3**:91–100.
8. Schmidt S, Christen P, Kiefer P, Vorholt JA. 2010. Functional investigation of methanol dehydrogenase-like protein XoxF in *Methylobacterium extorquens* AM1. *Microbiology* **156**:2575–2586.
9. Khadem AF, van Teeseling MCF, Jetten M, Op den Camp HJM, Pol A. 2012. Genomic and physiological analysis of carbon storage in the verrucomicrobial methanotroph “*Ca. Methylococcus*” SolV. *Front. Microbiol.* **3**:345.
10. Stein LY, Bringel F, DiSpirito AA, Han S, Jetten MS, Kalyuzhnaya MG, Kits KD, Klotz MG, Op den Camp HJ, Semrau JD, Vuilleumier S, Bruce DC, Cheng JF, Davenport KW, Goodwin L, Han S, Hauser L, Lajus A, Land ML, Lapidus A, Lucas S, Médigue C, Pitluck S, Woyke T. 2011. Genome sequence of the methanotrophic alphaproteobacterium *Methylocystis* sp strain Rockwell (ATCC 49242). *J. Bacteriol.* **193**:2668–2669.
11. Nyerges G, Han SK, Stein LY. 2010. Effects of ammonium and nitrite on growth and competitive fitness of cultivated methanotrophic bacteria. *Appl. Environ. Microbiol.* **76**:5648–5651.
12. Svenning MM, Hestnes AG, Warttinen I, Stein LY, Klotz MG, Kalyuzhnaya MG, Spang A, Bringel F, Vuilleumier S, Lajus A, Médigue C, Bruce DC, Cheng JF, Goodwin L, Ivanova N, Han J, Han CS, Hauser L, Held B, Land ML, Lapidus A, Lucas S, Nolan M, Pitluck S, Woyke T. 2011. Genome sequence of the Arctic methanotroph *Methylobacter tundripaludum* SV96. *J. Bacteriol.* **193**:6418–6419.
13. Boden R, Cunliffe M, Scanlan J, Moussard H, Kits KD, Klotz MG, Jetten MS, Vuilleumier S, Han J, Peters L, Mikhailova N, Teshima H, Tapia R, Kyrpides N, Ivanova N, Pagani I, Cheng JF, Goodwin L, Han C, Hauser L, Land ML, Lapidus A, Lucas S, Pitluck S, Woyke T, Stein L, Murrell JC. 2011. Complete genome sequence of the aerobic marine methanotroph *Methylomonas methanica* MC09. *J. Bacteriol.* **193**: 7001–7002.