Genome Sequence of the Obligate Methanotroph

*Methylosinus trichosporium* Strain OB3b

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*Methylosinus trichosporium* OB3b (for “oddball” strain 3b) is an obligate aerobic methane-oxidizing alphaproteobacterium that was originally isolated in 1970 by Roger Whittenbury and colleagues. This strain has since been used extensively to elucidate the structure and function of several key enzymes of methane oxidation, including both particulate and soluble methane monooxygenase (sMMO) and the extracellular copper chelator methanobactin. In particular, the catalytic properties of soluble methane monooxygenase from *M. trichosporium* OB3b have been well characterized in context with biodegradation of recalcitrant hydrocarbons, such as trichloroethylene. The sequence of the *M. trichosporium* OB3b genome is the first reported from a member of the Methyloccaceae family in the order Rhizobiales.

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Aerobic methanotrophic bacteria appear to be ubiquitous in the terrestrial and aquatic environment (9) and are a major biological sink for methane, the second most important greenhouse gas (10, 14, 15). Methanotrophic bacteria also have considerable potential for use in biotechnology (i.e., protein production) and in bioremediation due to the extensive substrate range of methane monooxygenase enzymes and amenability of these bacteria to large-scale cultivation (6, 8, 11, 14).

The genome of the obligate methanotrophic bacterium *Methylosinus trichosporium* OB3b (18) was sequenced, assembled, and annotated by the U.S. Department of Energy Joint Genome Institute (http://www.jgi.doe.gov/sequencing/). A quality draft of 44 contigs and 9 scaffolds was assembled from Roche 454-FLX, 454-std, 454-Titanium PE, and Illuma Solexa reads using Velvet (21). Automatic annotation was done using the Prokaryotic Dynamic Programming Genefinding Algorithm (PRODIGAL) (5). The *M. trichosporium* OB3b draft genome is 4.9 Mbp in size, contains 66% G+C, and encodes a single rRNA operon, a full complement of tRNA genes, and 4,503 predicted protein-encoding gene models. Sequence annotation and comparative genome analysis are under way with assistance from the Microscope annotation platform for annotation and comparative analysis of bacterial genomes at Genoscope (16).

Previously studied or predicted genes encoding enzymes and proteins involved in methane oxidation (methane oxidation inventory) were identified; these included both soluble and particulate methane monooxygenases, methanol dehydrogenase, proteins and enzymes involved in pyrroloquinoline quinone synthesis and tetrahydrofolate- and tetrahydromethanopterin-linked pathways, NAD-linked formate dehydrogenases, and hydrogenase. Genes encoding a putative nonribosomal polypeptide synthetase complex implicated in synthesis of peptides involved in metal uptake by methanotrophs were also identified (17). As observed for other alphaproteobacterial methanotrophs (2, 12), but in contrast to *Gammamethanotropha* (19), the *M. trichosporium* OB3b genome encodes the enzymes previously studied or predicted genes encoding enzymes and proteins involved in methane oxidation (methane oxidation inventory) were identified; these included both soluble and particulate methane monooxygenases, methanol dehydrogenase, proteins and enzymes involved in pyrroloquinoline quinone synthesis and tetrahydrofolate- and tetrahydromethanopterin-linked pathways, NAD-linked formate dehydrogenases, and hydrogenase. Genes encoding a putative nonribosomal polypeptide synthetase complex implicated in synthesis of peptides involved in metal uptake by methanotrophs were also identified (17). As observed for other alphaproteobacterial methanotrophs (2, 12), but in contrast to *Gammamethanotropha* (19), the *M. trichosporium* OB3b genome encodes the enzymes...
of a complete Embden-Meyerhoff glycolysis pathway and a closed tricarboxylic acid cycle, including α-ketoglutarate dehydrogenase. Whereas ribulose phosphokinase and other enzymes required for CO₂ fixation via the Calvin-Benson-Bassham cycle were found in the genome, genes encoding ribulose-bisphosphate carboxylase/oxygenase have not yet been identified (1).

As expected from several reports for methanotrophs and related members of the proteobacterial class Alphaproteobacteria (15), a full complement of genes encoding needed in vivo ammonia transport, and assimilation were identified. In addition, a gene cluster encoding the nsr response regulator and hybrid cluster (prismane) protein (13) was identified, suggesting operation of a pathway for hydroxylamine detoxification by reduction to ammonium. The M. trichosporium OB3b genome also encodes a cytochrome c'-alpha (pfam01322) in the cytochrom C2 superfamily (e0101610) (4) that has been implicated in reduction of NO to nitrous oxide in diverse alphaproteobacteria and beta-proteobacteria (3), supporting observed nitrous oxide production by this strain (7, 20). Genes that have not yet been conclusively identified in the inventory include those encoding proteins with the capacity to oxidize hydroxylamine to nitrite and reduce nitrite to NO. Also missing are the high-molecular-mass multiheme cytochromes observed in Methylococcus capsulatus Bath (17). These features may well be identified upon closure of the genome.

**Nucleotide sequence accession number.** The M. trichosporium OB3b genome sequence is available in GenBank under accession number ADVE00000000.

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**REFERENCES**


