JOURNAL OF BACTERIOLOGY, July 2010, p. 3840–3841 Vol. 192, No. 14
0021-9193/10/$12.00 doi:10.1128/JB.00506-10

Complete Genome Sequence of the Aerobic Facultative Methanotroph *Methylocella silvestris* BL2[▽]

Yin Chen,[1] Andrew Crombie,[1] M. Tanvir Rahman,[1] Svetlana N. Dedysh,[2] Werner Liesack,[3] Matthew B. Stott,[4] Maqsudul Alam,[5] Andreas R. Theisen,[1] J. Colin Murrell,[1] and Peter F. Dunfield[6]*

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom[1]; Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia[2]; Max-Planck-Institut für terrestrische Mikrobiologie, D-35043 Marburg, Germany[3]; GNS Science, Wairakei Research Institute, Taupo, New Zealand[4]; Department of Microbiology, University of Hawaii, Honolulu, Hawaii 96822[5]; and Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada[6]

Received 3 May 2010/Accepted 7 May 2010

***Methylocella silvestris* BL2 is an aerobic methanotroph originally isolated from an acidic forest soil in Germany. It is the first fully authenticated facultative methanotroph. It grows not only on methane and other one-carbon (C_1) substrates, but also on some compounds containing carbon-carbon bonds, such as acetate, pyruvate, propane, and succinate. Here we report the full genome sequence of this bacterium.**

Methylocella spp. are abundant in acidic soils and wetlands and help attenuate methane emissions from these habitats (2). They are unique in several ways compared to all other known aerobic methanotrophs. Notably, they lack extensive internal membrane systems and also appear to lack the particulate methane monooxygenase (pMMO) enzyme found in all other methanotrophs (6). Instead, they use only a soluble methane monooxygenase (sMMO) for methane oxidation. In addition, *Methylocella* spp. are not limited like other methanotrophs to growing on one-carbon (C_1) compounds but also utilize a number of multicarbon compounds (3). The genome of *Methylocella silvestris* BL2 (4) was sequenced, assembled, and annotated by the Joint Genome Institute (U.S. Department of Energy; http://www.jgi.doe.gov/sequencing/strategy.html). A total of 38,459 reads (~6× coverage), including 32,993 paired-end shotgun Sanger reads, 5,040 Roche 454 reads, and 580 finishing reads were included in the final assembly. Three lanes of Solexa data were used to polish the project.

The genome size is 4.3 Mbp. The G+C content is 63%. In total, 3,917 candidate genes were predicted and 99 pseudogenes were found. Functionality was assigned to 67.9% of the genes, while 30.9% of the genes could not be assigned any known function. Based on BLASTP searches against the KEGG (Kyoto Encyclopedia of Genes and Genomes) database, 3,413 out of 3,917 (87.1%) candidate genes have significant similarity to genes from *Proteobacteria*. Only 11 and 14 genes have best hits to genes from *Archaea* and *Eukarya*, respectively. All tRNA-encoding regions were identified, and two identical rRNA operons were found.

The absence of any *pmoCAB* genes encoding a pMMO enzyme that is present in all other genera of methanotrophs is now conclusively verified by the genome sequence. A complete operon encoding sMMO (*mmoXYBZDC*) was verified, as was a complete operon encoding methanol dehydrogenase (*mxaFJGIRSACKLDEH*) and all genes necessary for fixation of methane-derived carbon via the serine cycle. Genes encoding key enzymes in both the tetrahydrofolate and the tetrahydromethanopterin-mediated formaldehyde oxidation pathways were found.

M. silvestris can grow on two-carbon compounds, particularly acetate. Acetate kinase- and phosphotransacetylase-encoding genes are present, allowing acetate to be fed into the tricarboxylic acid (TCA) cycle. Genes encoding glyoxylate bypass enzymes (i.e., isocitrate lyase and malate synthase) have been identified. This pathway is essential for bacteria when growing on two-carbon compounds (1). The bacterium can also grow on C_3 and C_4 compounds, and a full gene set encoding enzymes of the TCA cycle is present, including genes encoding α-ketoglutarate dehydrogenase, which are lacking in some methanotrophs. Interestingly, a gene cluster encoding di-iron-containing multi-component propane monooxygenase is also present.

The genome sequence of *M. silvestris* is the first genome available for an alphaproteobacterial methanotroph. It joins the gammaproteobacterial methanotroph *Methylococcus capsulatus* Bath (7) and the verrucomicrobial methanotroph "*Methylacidiphilum infernorum*" (5). More detailed analyses of the genome as well as comparative analysis with obligate methanotrophs will provide deeper insight into the metabolism of this fascinating bacterium.

Nucleotide sequence accession number. The genome sequence and annotation have been deposited in GenBank under accession no. CP001280.

* Corresponding author. Mailing address: Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, Alberta T2N 1N4 Canada. Phone: 403-220-2469. Fax: 403-289-9311. E-mail: pfdunfie@ucalgary.ca.

▽ Published ahead of print on 14 May 2010.

This work was performed under the auspices of the U.S. Department of Energy's Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Livermore National Laboratory, under contract no. W-7405-Eng-48, Lawrence Berkeley National Laboratory under contract no. DE-AC02-

05CH11231, and Los Alamos National Laboratory under contract no. DE-AC02-06NA25396.

REFERENCES

1. **Chung, T., D. J. Klumpp, and D. C. LaPorte.** 1988. Glyoxylate bypass operon of *Escherichia coli*: cloning and determination of the functional map. J. Bacteriol. **170:**386–392.
2. **Dedysh, S. N., and P. F. Dunfield.** 2010. Facultative methane oxidizers, p. 1967–1976. *In* K. N. Timmis (ed.), Handbook of hydrocarbon and lipid microbiology. Springer-Verlag, Berlin, Germany.
3. **Dedysh, S. N., C. Knief, and P. F. Dunfield.** 2005. *Methylocella* species are facultatively methanotrophic. J. Bacteriol. **187:**4665–4670.
4. **Dunfield, P. F., V. N. Khmelenina, N. E. Suzina, Y. A. Trotsenko, and S. N. Dedysh.** 2003. *Methylocella silvestris* sp. nov., a novel methanotrophic bacterium isolated from an acidic forest cambisol. Int. J. Syst. Evol. Microbiol. **53:**1231–1239.
5. **Hou, S., K. S. Makarova, J. H. W. Saw, P. Senin, B. V. Ly, Z. Zhou, Y. Ren, J. Wang, M. Y. Galperin, M. V. Omelchenko, Y. I. Wolf, N. Yutin, E. V. Koonin, M. B. Stott, B. W. Mountain, M. Crowe, A. V. Smirnova, P. F. Dunfield, L. Feng, L. Wang, and M. Alam.** 2008. Complete genome sequence of the extremely acidophilic methanotroph isolate V4, "*Methylacidiphilum infernorum*," a representative of the bacterial phylum *Verrucomicrobia*. Biol. Direct **3:**26.
6. **Theisen, A. R., M. H. Ali, S. Radajewski, M. G. Dumont, P. F. Dunfield, I. R. McDonald, S. N. Dedysh, C. B. Miguez, and J. C. Murrell.** 2005. Regulation of methane oxidation in the facultative methanotroph *Methylocella silvestris* BL2. Mol. Microbiol. **58:**682–692.
7. **Ward, N., O. Larsen, J. Sakwa, L. Bruseth, H. Khouri, A. S. Durkin, G. Dimitrov, L. Jiang, D. Scanlan, K. H. Kang, M. Lewis, K. E. Nelson, B. A. Methé, M. Wu, J. F. Heidelberg, I. T. Paulsen, D. Fouts, J. Ravel, H. Tettelin, Q. Ren, T. Read, R. T. DeBoy, R. Seshadri, S. L. Salzberg, H. B. Jensen, N. K. Birkeland, W. C. Nelson, R. J. Dodson, S. H. Grindhaug, I. Holt, I. Eidhammer, I. Jonasen, S. Vanaken, T. Utterback, T. V. Feldblyum, C. M. Fraser, J. R. Lillehaug, and J. A. Eisen.** 2004. Genomic insights into methanotrophy: the complete genome sequence of *Methylococcus capsulatus* (Bath). PLoS Biol. **2:**e303.