

# Draft Genome Sequence of *Methyloferula stellata* AR4, an Obligate Methanotroph Possessing Only a Soluble Methane Monooxygenase

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***Methyloferula stellata* AR4 is an aerobic acidophilic methanotroph, which, in contrast to most known methanotrophs but similar to *Methylocella* spp., possesses only a soluble methane monooxygenase. However, it differs from *Methylocella* spp. by its inability to grow on multicarbon substrates. Here, we report the draft genome sequence of this bacterium.**

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*Methyloferula stellata* AR4 is a methanotroph of the *Alphaproteobacteria* family *Beijerinckiaceae*. It is a typical inhabitant of acidic wetlands and soils (1). It represents only the second known genus of methanotrophs (after *Methylocella*) lacking a particulate methane monooxygenase (pMMO) and an extensive intracytoplasmic membrane system. *Methyloferula* spp. and *Methylocella* spp. possess only a soluble methane monooxygenase (sMMO) and share some phenotypic characteristics, such as tolerance to low pH and the ability to fix dinitrogen. However, they differ with regard to their substrate utilization patterns. *Methylocella* species are facultative methanotrophs, which, in addition to C<sub>1</sub> compounds, utilize acetate and several other organic acids, ethanol, and some short-chain alkanes (2–4). In contrast, *M. stellata* grows only on methane and methanol (1).

The draft genome sequence was generated at the Department of Energy (DOE) Joint Genome Institute (JGI) using Illumina technology (5). The combination of short-insert (insert size, ~250 bp) and long-insert (~9,500 bp) paired-end libraries produced 3,088 Mb of data (see <http://www.jgi.doe.gov/>). These were assembled with AllPaths version r41554 and computationally shredded into 10-kb overlapping fake reads (6). The Illumina data were also assembled with Velvet version 1.1.05 (7), computationally shredded into 1.5-kb overlapping fake reads, reassembled with Velvet, and shredded into 1.5-kb overlapping fake reads. The fake reads from the AllPaths and two Velvet assemblies, as well as a subset of the Illumina cross-linking and immunoprecipitation sequencing (CLIP) paired-end reads, were assembled using parallel Phrap version 4.24 (High Performance Software, LLC). Possible misassemblies were corrected with manual editing in Consed (8–10). The total estimated size of the *M. stellata* AR4 genome is 4.24 Mb (coverage, 735×), with an average G+C content of 59.5%. A single rRNA operon, 46 tRNAs, and 3,967 predicted protein-coding genes were identified.

The absence of pMMO-encoding genes and the presence of an

operon encoding sMMO (*mmoXYBZDC*) are unique features shared only by *Methyloferula stellata* and *Methylocella* species (11). An additional soluble diiron monooxygenase, i.e., propane monooxygenase, which is present in *Methylocella silvestris* BL2 (3, 11), is lacking in *M. stellata*. Strain AR4 contains a large array of genes encoding various alcohol dehydrogenase quinoproteins, i.e., one MxaFI- methanol dehydrogenase (MDH), five XoxF-MDHs, and one alcohol dehydrogenase type 6a quinoprotein (12). Genes involved in tetrahydromethanopterin-linked C<sub>1</sub> transfer and formate oxidation were also identified. Similarly to other *Beijerinckiaceae* methanotrophs (13), the genome of *M. stellata* contains the complete set of genes for the function of the Calvin-Benson-Bassham cycle and the serine pathway for carbon assimilation, as well as genes encoding enzymes of the tricarboxylic acid cycle. It lacks the ethylmalonyl-coenzyme A (CoA) pathway for glyoxylate regeneration but possesses a glyoxylate bypass. The number of membrane transporters in *Methyloferula stellata* AR4 is nearly the same as in *Methylocella silvestris* BL2 (13); *M. silvestris* BL2 however, possesses an acetate/glycolate transporter that is lacking in strain AR4. The genes involved in N<sub>2</sub> fixation are organized as in *Beijerinckia indica* (14) into two genomic islands, with one additional *nifK* gene homologue located outside these islands.

**Nucleotide sequence accession number.** The *M. stellata* AR4 genome sequence was deposited in GenBank/EMBL under the accession no. [ARWA000000000](https://www.ncbi.nlm.nih.gov/nuccore/ARWA000000000).

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## REFERENCES

- Vorobev AV, Baani M, Doronina NV, Brady AL, Liesack W, Dunfield PF, Dedysh SN. 2011. *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monooxygenase. *Int J Syst Evol Microbiol* 61:2456–2463. <http://dx.doi.org/10.1099/ijms.0.028118-0>.
- Dedysh SN, Knief C, Dunfield PF. 2005. *Methylocella* species are facultatively methanotrophic. *J Bacteriol* 187:4665–4670. <http://dx.doi.org/10.1128/JB.187.13.4665-4670.2005>.
- Crombie AT, Murrell JC. 2014. Trace-gas metabolic versatility of the facultative methanotroph *Methylocella silvestris*. *Nature* 510:148–151. <http://dx.doi.org/10.1038/nature13192>.
- Dunfield PF, Dedysh SN. 2014. *Methylocella*: a gourmand among methanotrophs. *Trends Microbiol* 22:368–369. <http://dx.doi.org/10.1016/j.tim.2014.05.004>.
- Bennett S. 2004. Solexa Ltd. *Pharmacogenomics* 5:433–438. <http://dx.doi.org/10.1517/14622416.5.4.433>.
- Butler J, MacCallum I, Kleber M, Shlyakhter IA, Belmonte MK, Lander ES, Nusbaum C, Jaffe DB. 2008. ALLPATHS: *de novo* assembly of whole-genome shotgun microreads. *Genome Res* 18:810–820. <http://dx.doi.org/10.1101/gr.7337908>.
- 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>.
- Ewing B, Green P. 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Res* 8:186–194.
- Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using Phred. I. Accuracy assessment. *Genome Res* 8:175186–185. <http://dx.doi.org/10.1101/gr.8.3.175>.
- Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. *Genome Res* 8:195–202. <http://dx.doi.org/10.1101/gr.8.3.195>.
- Chen Y, Crombie A, Rahman MT, Dedysh SN, Liesack W, Stott MB, Alam M, Theisen AR, Murrell JC, Dunfield PF. 2010. Complete genome sequence of the aerobic facultative methanotroph *Methylocella silvestris* BL2. *J Bacteriol* 192:3840–3841. <http://dx.doi.org/10.1128/JB.00506-10>.
- Keltjens JT, Pol A, Reimann J, Op den Camp HJM. 2014. PQQ-dependent methanol dehydrogenases: rare-earth elements make a difference. *Appl Microbiol Biotechnol* 98:6163–6183. <http://dx.doi.org/10.1007/s00253-014-5766-8>.
- Tamas I, Smirnova AV, He Z, Dunfield PF. 2014. The (d)evolution of methanotrophy in the *Beijerinckiaceae*—a comparative genomics analysis. *ISME J* 8:369–382. <http://dx.doi.org/10.1038/ismej.2013.145>.
- Tamas I, Dedysh SN, Liesack W, Stott MB, Alam M, Murrell JC, Dunfield PF. 2010. Complete genome sequence of *Beijerinckia indica* subsp. *indica*. *J Bacteriol* 192:4532–4533. <http://dx.doi.org/10.1128/JB.00656-10>.