

# Complete Genome Sequence of the Aerobic Marine Methanotroph *Methylomonas methanica* MC09

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***Methylomonas methanica* MC09 is a mesophilic, halotolerant, aerobic, methanotrophic member of the Gammaproteobacteria, isolated from coastal seawater. Here we present the complete genome sequence of this strain, the first available from an aerobic marine methanotroph.**

*Methylomonas methanica* (11, 21) is one of four recognized species within the genus *Methylomonas* in the Gammaproteobacteria, which includes *M. aurantiaca* (3, 12), *M. fodinarum* (3, 12), and *M. scandinavica* (13, 14). Several other *Methylomonas* species without validly published names have also been described, including “*M. clara*” (8) and “*M. rubra*” (18). All members of the genus use methane as the sole carbon and energy source. The majority of known strains were obtained from terrestrial environments; however, *M. methanica* MC09 was isolated from a methane enrichment culture inoculated with seawater obtained from the coast of Penarth, United Kingdom (lat 51.43, long –3.17) (M. Cunliffe and J. C. Murrell, unpublished data). *Methylomonas* spp. are prevalent in various marine and estuarine environments (5, 9, 16, 19, 20). The complete genome sequence of *M. methanica* MC09 is the first available for a marine methanotroph, providing insights into methane cycling in marine environments.

The genome (5.05 Mbp) of *M. methanica* MC09 was assembled using VELVET (22) and Newbler from an Illumina GAii (2) shotgun library (74,177,086 reads; 2.67 Gbp) and 454 Titanium (15) standard (215,708 reads) and paired-end (154 Mbp) libraries representing 24.3× coverage. Gaps were closed by PCR and Bubble PCR primer walks (350 reactions and 1 shatter library) using Consed (7). The genome is a single circular replicon with 4,494 candidate protein-encoding genes, as predicted by Prodigal (10) and GenePrimp (17). The mean GC content of the sequence was 51.3 mol%.

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Synthetic pathways for tRNAs of all 20 structural amino acids were accounted for, along with a single rRNA operon. Three terminal oxidases were predicted: *aa<sub>3</sub>*, *o*-quinol, and *bd*-quinol.

All genes for the 2-keto-3-deoxy-6-phosphogluconate (KDPG) aldolase variant of the ribulose monophosphate (RuMP) pathway of formaldehyde fixation were predicted, consistent with experimental data for *Methylomonas* spp. (1). All genes of the pentose phosphate and Embden-Meyerhof-Parnas pathways were predicted. Genes for all enzymes of Krebs' cycle, with the exception of fumarase, were predicted. Genes for RubisCO were not found. The *mxajFGIRSACKLDEK* cluster encoding methanol dehydrogenase was predicted, along with the cluster *pqqBCDE* for biosynthesis of the cofactor pyrroloquinoline quinone. Both particulate (*pmoCAB*) and soluble (*mnoXYBZDCGR*) methane monooxygenases were predicted. Acetate kinase and acetyl coenzyme A synthase were predicted, potentially allowing C<sub>2</sub>-compound assimilation.

All genes required for dinitrogen fixation were predicted, as were those for nitrate/nitrite transport (*nasFED*), ammonification (*nasCA*, *nasB*, and *nirBD*), direct ammonium uptake (*amtB*), and nitrogen assimilation (*ghnA*, *gltB*, and *ald*). Urea metabolism genes were predicted (carbamoyl phosphate synthase, *carA* and *carB*; urease, *ureABC*); however, neither a complete urea cycle (lacking the arginase gene) nor functional urease (lacking accessory genes) is present. Genes for nitrite reduction (*nirS*) and nitric oxide reduction (*norB*) were predicted. A gene encoding cytochrome P460 was predicted, indicating a potential for hydroxylamine detoxification (4, 6).

The information provided in the complete genome sequence of *M. methanica* MC09 will enable further studies of the metabolism of this and other methanotrophic bacteria. These data also provide the first overview of the metabolic diversity of a marine methanotroph.

**Nucleotide sequence accession number.** The nucleotide sequence of the genome has been deposited in DDBJ/EMBL/GenBank under accession no. CP002738.

The sequencing was carried out by the DOE Joint Genome Institute with support from their community sequencing program, and the genome is the first of a series of 18 genomes of methanotrophic *Bacteria* to be analyzed by the Organization for Methanotroph Genome Analysis (OMeGA). The work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of Science of the U.S. Department of Energy under contract no. DE-AC02-05CH11231. L.S. was supported by a grant from the NSERC. M.G.K. was supported by incentive funds from the University of Louisville. M.C. and R.B. were supported by the NERC.

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