

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

Rich Boden,^{1*} Michael Cunliffe,^{1†} Julie Scanlan,¹ H el ene Moussard,¹ K. Dimitri Kits,² Martin G. Klotz,³ Mike S. M. Jetten,⁴ St ephane Vuilleumier,⁵ James Han,⁶ Lin Peters,⁶ Natalia Mikhailova,⁶ Hazuki Teshima,⁷ Roxanne Tapia,^{6,7} Nikos Kyrpides,⁶ Natalia Ivanova,⁶ Ioanna Pagani,⁶ Jan-Fang Cheng,⁶ Lynne Goodwin,⁷ Cliff Han,^{6,7} Loren Hauser,^{6,8} Miriam L. Land,^{6,8} Alla Lapidus,⁶ Susan Lucas,⁶ Sam Pitluck,⁶ Tanja Woyke,⁶ Lisa Stein,² and J. Colin Murrell¹

School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, United Kingdom¹; Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9, Canada²; Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina³; Faculty of Science, Radboud University Nijmegen, Nijmegen, The Netherlands⁴; UMR 7156 CRNS, Universit e de Strasbourg, F-67000 Strasbourg, France⁵; US DOE Joint Genome Institute, Walnut Creek, California 94598⁶; Los Alamos National Laboratory, Joint Genome Institute, Biosciences Division Genome Science B6, Los Alamos, New Mexico 87545⁷; and Oak Ridge National Laboratory, Bioscience Division, Oak Ridge, Tennessee 37831⁸

Received 27 September 2011/Accepted 10 October 2011

***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seawater obtained from the coast of Penarth, United Kingdom (lat 51.43, long –3.17) (M. Cunliffe and J. C. Murrell, unpublished data).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%.

* Corresponding author. Mailing address: School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, United Kingdom. Phone: 44 0 24 7652 2557. Fax: 44 0 24 7652 3568. E-mail: rich.boden@warwick.ac.uk.

† Present address: Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth PL1 2PB, United Kingdom.

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*₃, *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 *mx**A**FJGIRSACKLDEK* 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 C₂-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.

We thank Marina Kalyuzhnaya for helpful comments on the manuscript.

REFERENCES

1. Anthony, C. 1982. The biochemistry of methylotrophs. Academic Press, New York, NY.
2. Bennett, S. 2004. Solexa Ltd. Pharmacogenomics **5**:433–438.
3. Bowman, J. P., L. I. Sly, J. M. Cox, and A. C. Hayward. 1990. *Methylomonas fodinarum* sp. nov. and *Methylomonas aurantiaca* sp. nov.: two closely related type I obligate methanotrophs. Syst. Appl. Microbiol. **13**:278–286.
4. Campbell, M. A., et al. 2011. Model of the molecular basis for hydroxylamine oxidation and nitrous oxide production in methanotrophic bacteria. FEMS Microbiol. Lett. **322**:82–89.
5. Cunliffe, M., et al. 2008. Phylogenetic and functional gene analysis of the bacterial and archaeal communities associated with the surface microlayer of an estuary. ISME J. **2**:776–789.
6. Elmore, B. O., D. J. Bergmann, M. G. Klotz, and A. B. Hooper. 2007. Cytochromes P460 and *c'*- β : a new family of high-spin cytochromes *c*. FEBS Lett. **581**:911–916.
7. Gordon, D., C. Abajian, and P. Green. 1998. Consed: a graphical tool for sequence finishing. Genome Res. **8**:195–202.
8. Hohenloser, W., F. Lingens, and P. Pr ave. 1978. Characterization of a new methylotrophic strain, *Methylomonas clara*. Eur. J. Appl. Microbiol. Biotechnol. **6**:167–179.
9. Holmes, A., N. J. P. Owens, and J. C. Murrell. 1995. Detection of novel marine methanotrophs using phylogenetic and functional gene probes after methane enrichment. Microbiology **141**:1947–1955.
10. Hyatt, D., et al. 2010. Prodigal prokaryotic dynamic programming gene-finding algorithm. BMC Bioinformatics **11**:119.
11. International Journal of Systematic Bacteriology. 1984. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 15. Int. J. Syst. Bacteriol. **34**:355–357.
12. International Journal of Systematic Bacteriology. 1990. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 35. Int. J. Syst. Bacteriol. **40**:470–471.
13. International Journal of Systematic and Evolutionary Microbiology. 2000. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. List no. 74. Int. J. Syst. Evol. Microbiol. **50**:949–950.
14. Kalyuzhnaya, M. G., et al. 1999. *Methylomonas scandinavica* sp. nov., a new methanotrophic psychrotrophic bacterium isolated from deep igneous rock ground water of Sweden. Syst. Appl. Microbiol. **22**:565–572.
15. Margulies M., et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature **437**:376–380.
16. McDonald, I. R., K. Smith, and M. E. Lidstrom. 2005. Methanotrophic populations in estuarine sediment from Newport Bay, California. FEMS Microbiol. Lett. **250**:287–293.
17. Pati, A., et al. 2010. GenePRIMP: a gene prediction improvement pipeline for microbial genomes. Nat. Methods **7**:455–457.
18. Romanovskaya, V. A., P. V. Rokitko, S. O. Shilin, and Y. R. Malashenko. 2006. Emended description of *Methylomonas rubra* sp. nov. Mikrobiologiya **75**:689–693.
19. Sieburth, J. M., P. W. Johnson, V. M. Church, and D. C. Laux. 1993. *C*₁ bacteria in the water column of Chesapeake Bay, USA. 3. Immunological relationships of the type species of marine monomethylamine-oxidizing and methane-oxidizing bacteria to wild estuarine and oceanic cultures. Mar. Ecol. Prog. Ser. **95**:91–102.
20. Upstill-Goddard, R. C. 2006. Air-sea gas exchange in the coastal zone. Estuar. Coast. Shelf Sci. **70**:388–404.
21. Whittenbury, R., and N. R. Krieg. 1984. Family *Methylococcaceae*, p. 256–261. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology. Williams & Wilkins, Baltimore, MD.
22. Zerbino, D. R., and E. Birney. 2008. Velvet: algorithms for *de novo* short read assembly using de Bruijn graphs. Genome Res. **18**:821–829.