Draft genome sequence of the volcano-inhabiting thermoacidophilic methanotroph

*Methylacidiphilum fumariolicum* strain SolV

Ahmad F. Khadem¹, Adam S. Wieczorek¹, Arjan Pol¹, Stéphane Vuilleumier², Harry R. Harhangi¹, Peter F. Dunfield³, Marina G. Kalyuzhnaya⁴, J. Colin Murrell⁵, Kees-Jan Francoijs⁶, Henk G. Stunnenberg⁶, Lisa Y. Stein⁷, Alan A. DiSpirito⁸, Jeremy D. Semrau⁹, Aurélie Lajus¹⁰, Claudine Médigue¹⁰, Martin G. Klotz¹¹, Mike S.M. Jetten¹, Huub J.M. Op den Camp¹*

¹Department of Microbiology, IWW, Radboud University Nijmegen, Heyendaalseweg 135, NL-6525 AJ, Nijmegen, the Netherlands
²Université de Strasbourg, UMR 7156 CNRS, 67000 Strasbourg, France
³Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, Alberta, Canada, T2N 1N4
⁴Department of Microbiology, University of Washington, Seattle WA 98195 USA,
⁵School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK
⁶Department of Molecular Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen, Geert Grootenplein-Zuid 26, NL-6525 GA, Nijmegen, the Netherlands
⁷Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9 Canada
⁸Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University Ames, Iowa 50011, USA
⁹Department of Civil and Environmental Engineering, The University of Michigan, 1351 Beal Avenue, Ann Arbor, Michigan 48109-2125, USA
¹⁰Laboratoire d’Analyses Bioinformatiques pour la Génomique et le Métabolisme (LABGeM), Genoscope-IG-CEA, 91057 Evry, France.
¹¹Department of Biology, University of North Carolina, Charlotte, NC 28223, USA

* Corresponding author.

Dr. H.J.M. Op den Camp
Department of Microbiology, IWW
Radboud University Nijmegen
Heyendaalseweg 135,
NL-6525 AJ, Nijmegen, the Netherlands
Tel. +31 24 3652657
Fax +31 24 3652830
E-mail: h.opdencamp@science.ru.nl
The draft genome of *Methylacidiphilum fumariolicum* SolV, a thermoacidophilic methanotroph of the Verrucomicrobia phylum is presented. Annotation revealed pathways for one-carbon, nitrogen, and hydrogen catabolism and respiration together with central metabolic pathways. The genome encodes three orthologues of particulate methane monooxygenases and helps to understand methane cycling in volcanic environments.

Isolation (14) and genome sequencing of strain SolV led to the proposal that *Methylacidiphilum fumariolicum* be one of three proposed species within the genus *Methylacidiphilum* (13), together with *M. infernorum* (strain V4)(4), and *M. kamchatkensis* (strain Kam1)(7). All three strains were isolated from acidic volcanic areas and are well adapted to the harsh volcanic environment (13, 14), being able to thrive at very low methane and oxygen concentrations and pH values as low as 1.

The high-quality draft genome of *M. fumariolicum* SolV (109 contigs) was assembled from Illumina and Roche 454 reads using CLCbio (http://www.clcbio.com) and manual adjustments. The draft genome is 2.36 Mbp in size with a GC content of 40.9%. Auto-annotation was performed based on comparison to public databases using the MicroScope platform (Genoscope, France) (17), which identified 2283 protein-encoding gene models. For 623 of these, full-length homologs (>80% identity at the protein level) were present in the complete genome of *M. infernorum* V4 (6), with 619 of them organised in synteny in the two strains. Biosynthetic pathways and tRNA’s of all 20 amino acids were present together with a single rRNA operon.

Key genes for the ribulose monophosphate pathway and the serine cycle were absent. However, genes encoding the Calvin-Benson-Bassham cycle enzymes were present, supporting physiological studies (9). The genome encodes all three central pathways: Embden-Meyerhof-Parnas glycolytic pathway, the pentose phosphate pathway and the tricarboxylic acid cycle. Genes encoding keto-deoxy-gluconate catabolism of the Entner-Doudoroff pathway were absent. Three particulate methane monooxygenase operons (*pmoCAB*) were predicted, while genes encoding soluble methane monooxygenase were not found. The *mxaFI* genes encoding methanol dehydrogenase (2, 3) were absent, but a homologous *xoxFJG* gene cluster and a *ppqABCDEF* operon for the biosynthesis of the cofactor pyroloquinoline quinone were detected. H₄MPT-linked C1-transfer genes are not
present. The H₄Folate-linked pathway inventory includes metF, folD and ftfL genes. Neither mtdA, fch or purU were found. Genes encoding NAD-linked formate dehydrogenase (fdsABG) were identified (12). Should the identified genes encoding acetate kinase and acetyl-coenzyme A synthase prove functional, strain SolV may be able to assimilate C2 compounds, and thus be a facultative methanotroph (15). The presence of a hydrogenase gene cluster points towards possible chemolithotrophic growth or the use of hydrogen to provide reducing equivalents for methane oxidation (5). A complex IV-type heme-copper oxidase gene cluster possibly encodes the terminal cytochrome c oxidase.

Strain SolV was able to grow with ammonium, nitrate or dinitrogen gas as nitrogen source (8, 14). Coincidentally, genes were predicted for direct ammonium uptake (amtB) and assimilation (e.g. glutamine synthase, glnA; glutamate synthase, gltB; alanine dehydrogenase, ald) as well as for urea metabolism. As in most other methanotrophs, however, the urea cycle is incomplete (1). A full complement of genes for dinitrogen fixation, nitrate/nitrite transport and assimilation was also found. In addition, genes for nitrite reduction (nirK) and nitric oxide reduction (norB, norC), were identified but the inventory to encode nitrous oxide reduction was missing. A haoAB gene cluster encoding hydroxylamine oxidase was identified, suggesting the capability of nitrification and nitrosative stress handling (10, 11, 16).

**Nucleotide sequence accession number.** The nucleotide genome sequence of *M. fumariolicum* SolV has been deposited in the European Nucleotide Archive (ENA) under accession numbers CAHT01000001 to CAHT01000109.

**ACKNOWLEDGMENTS**

The work of A.F. Khadem was supported by Mosaic grant 62000583 from the Dutch Organization for Scientific Research-NWO. A.S. Wieczorek and M.S.M. Jetten were supported by grant 232937 from the European Research Council.

**REFERENCES**


