

## PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is an author's version which may differ from the publisher's version.

For additional information about this publication click this link.

<http://hdl.handle.net/2066/93817>

Please be advised that this information was generated on 2017-12-06 and may be subject to change.

1 GENOME ANNOUNCEMENT

2

3 **Draft genome sequence of the volcano-inhabiting thermoacidophilic methanotroph**  
4 ***Methylacidiphilum fumariolicum* strain SoIV**

5

6 Ahmad F. Khadem<sup>1</sup>, Adam S. Wiczorek<sup>1</sup>, Arjan Pol<sup>1</sup>, Stéphane Vuilleumier<sup>2</sup>, Harry R. Harhangi<sup>1</sup>,  
7 Peter F. Dunfield<sup>3</sup>, Marina G. Kalyuzhnaya<sup>4</sup>, J. Colin Murrell<sup>5</sup>, Kees-Jan Francoijs<sup>6</sup>, Henk G.  
8 Stunnenberg<sup>6</sup>, Lisa Y. Stein<sup>7</sup>, Alan A. DiSpirito<sup>8</sup>, Jeremy D. Semrau<sup>9</sup>, Aurélie Lajus<sup>10</sup>, Claudine  
9 Médigue<sup>10</sup>, Martin G. Klotz<sup>11</sup>, Mike S.M. Jetten<sup>1</sup>, Huub J.M. Op den Camp<sup>1\*</sup>

10

11 <sup>1</sup>Department of Microbiology, IWW, Radboud University Nijmegen, Heyendaalseweg 135, NL-6525 AJ,  
12 Nijmegen, the Netherlands

13 <sup>2</sup>Université de Strasbourg, UMR 7156 CNRS, 67000 Strasbourg, France

14 <sup>3</sup>Department of Biological Sciences, University of Calgary, 2500 University Drive NW,  
15 Calgary, Alberta, Canada, T2N 1N4

16 <sup>4</sup>Department of Microbiology, University of Washington, Seattle WA 98195 USA,

17 <sup>5</sup>School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL, UK

18 <sup>6</sup>Department of Molecular Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University  
19 Nijmegen, Geert Grooteplein-Zuid 26, NL-6525 GA, Nijmegen, the Netherlands

20 <sup>7</sup>Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2E9 Canada

21 <sup>8</sup>Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University Ames, Iowa 50011,  
22 USA

23 <sup>9</sup>Department of Civil and Environmental Engineering, The University of Michigan, 1351 Beal Avenue, Ann  
24 Arbor, Michigan 48109-2125, USA

25 <sup>10</sup>Laboratoire d'Analyses Bioinformatiques pour la Génomique et le Métabolisme (LABGeM), Genoscope-IG-  
26 CEA, 91057 Evry, France.

27 <sup>11</sup>Department of Biology, University of North Carolina, Charlotte, NC 28223, USA

28

29

30 **\* Corresponding author.**

31 Dr. H.J.M. Op den Camp

32 Department of Microbiology, IWW

33 Radboud University Nijmegen

34 Heyendaalseweg 135,

35 NL-6525 AJ, Nijmegen, the Netherlands

36 Tel. +31 24 3652657

37 Fax +31 24 3652830

38 E-mail: [h.opdencamp@science.ru.nl](mailto:h.opdencamp@science.ru.nl)

39

40 [ABSTRACT]

41 The draft genome of *Methylophilum fumariolicum* SolV, a thermoacidophilic  
42 methanotroph of the Verrucomicrobia phylum is presented. Annotation revealed pathways for  
43 one-carbon, nitrogen, and hydrogen catabolism and respiration together with central  
44 metabolic pathways. The genome encodes three orthologues of particulate methane  
45 monooxygenases and helps to understand methane cycling in volcanic environments.

46

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

53 The high-quality draft genome of *M. fumariolicum* SolV (109 contigs) was assembled  
54 from Illumina and Roche 454 reads using CLCbio (<http://www.clcbio.com>) and manual  
55 adjustments. The draft genome is 2.36 Mbp in size with a GC content of 40.9%. Auto-  
56 annotation was performed based on comparison to public databases using the MicroScope  
57 platform (Genoscope, France) (17), which identified 2283 protein-encoding gene models. For  
58 623 of these, full-length homologs (>80% identity at the protein level) were present in the  
59 complete genome of *M. infernorum* V4 (6), with 619 of them organised in synteny in the two  
60 strains. Biosynthetic pathways and tRNA's of all 20 amino acids were present together with a  
61 single rRNA operon.

62 Key genes for the ribulose monophosphate pathway and the serine cycle were absent.  
63 However, genes encoding the Calvin-Benson-Bassham cycle enzymes were present,  
64 supporting physiological studies (9). The genome encodes all three central pathways:  
65 Embden-Meyerhof-Parnas glycolytic pathway, the pentose phosphate pathway and the  
66 tricarboxylic acid cycle. Genes encoding keto-deoxy-gluconate catabolism of the Entner-  
67 Doudoroff pathway were absent. Three particulate methane monooxygenase operons  
68 (*pmoCAB*) were predicted, while genes encoding soluble methane monooxygenase were not  
69 found. The *mxoFI* genes encoding methanol dehydrogenase (2, 3) were absent, but a  
70 homologous *xoxFJG* gene cluster and a *pqqABCDEF* operon for the biosynthesis of the  
71 cofactor pyroloquinoline quinone were detected. H<sub>4</sub>MPT-linked C1-transfer genes are not

72 present. The H<sub>4</sub>Folate-linked pathway inventory includes *metF*, *fold* and *fffL* genes. Neither  
73 *mtaA*, *fch* or *purU* were found. Genes encoding NAD-linked formate dehydrogenase  
74 (*fdsABG*) were identified (12). Should the identified genes encoding acetate kinase and acetyl-  
75 coenzyme A synthase prove functional, strain SolV may be able to assimilate C<sub>2</sub> compounds,  
76 and thus be a facultative methanotroph (15). The presence of a hydrogenase gene cluster  
77 points towards possible chemolithotrophic growth or the use of hydrogen to provide reducing  
78 equivalents for methane oxidation (5). A complex IV-type heme-copper oxidase gene cluster  
79 possibly encodes the terminal cytochrome c oxidase.

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

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

92

### 93 **ACKNOWLEDGMENTS**

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

97

### 98 **REFERENCES**

- 99 1. **Boden R, Cunliffe, M, Scanlan J, Moussard H, Kits KD, Klotz MG, Jetten SM,**  
100 **Vuilleumier S, Han, J, Peters L, Mikhailova N, Teshima H, Tapia R, Kyrpides N, Ivanova**  
101 **N, Pagani I, Cheng J-F, Goodwin L, Han C, Hauser L, Land ML, Lapidus A, Lucas S,**  
102 **Pitluck S, Woyke T, Stein L, Murrell JC.** 2011. Complete genome sequence of aerobic marine  
103 methanotroph *Methylomonas methanica* MC09. J. Bacteriol. **193**:7001-7002.

- 104 2. **Chen Y, Crombie A, Rahman MT, Dedysh SN, Liesack W, Stott MB, Alam M, Theisen**  
105 **AR, Murrell JC, Dunfield PF.** 2010. Complete genome sequence of the aerobic facultative  
106 methanotroph *Methylocella silvestris* BL2. J. Bacteriol. **192**:3840-3841.
- 107 3. **Chistoserdova L, Kalyuzhnaya MG, Lidstrom ME.** 2009. The expanding world of  
108 methylotrophic metabolism. Annu. Rev. Microbiol. **63**:477-499.
- 109 4. **Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S, Ly B, Saw JH, Zhou Z,**  
110 **Ren Y, Wang J, Mountain BW, Crowe MA, Weatherby TM, Bodelier PLE, Liesack W,**  
111 **Feng L, Wang L, Alam M.** 2007. Methane oxidation by an extremely acidophilic bacterium of  
112 the phylum Verrucomicrobia. Nature **450**:879-882.
- 113 5. **Hanczár T, Csáki R, Bodrossy L, Murrell JC, Kovács K.** 2002. Detection and localization of  
114 two hydrogenases in *Methylococcus capsulatus* (Bath) and their potential role in methane  
115 metabolism. Arch. Microbiol. **177**:167-172.
- 116 6. **Hou S, Makarova KS, Saw JHW, Senin P, Ly BV, Zhou Z, Ren Y, Wang J, Galperin MY,**  
117 **Omelchenko MV, Wolf YI, Yutin N, Koonin EV, Stott MB, Mountain BW, Crowe M,**  
118 **Smirnova AV, Dunfield PF, Feng L, Wang L, Alam M.** 2008. Complete genome sequence of  
119 the extremely acidophilic methanotroph isolate V4, "*Methylacidiphilum infernorum*", a  
120 representative of the bacterial phylum Verrucomicrobia. Biol. Direct **3**:26.
- 121 7. **Islam T, Jensen S, Reigstad LJ, Larsen O, Birkeland NK.** 2008. Methane oxidation at 55°C  
122 and pH 2 by a thermoacidophilic bacterium belonging to the Verrucomicrobia phylum. Proc.  
123 Natl. Acad. Sci. USA **105**:300-304.
- 124 8. **Khadem AF, Pol A, Jetten MSM, Op den Camp HJM.** 2010. Nitrogen fixation by the  
125 verrucomicrobial methanotroph *Methylacidiphilum fumariolicum* SolV. Microbiology **156**:1052-  
126 1059.
- 127 9. **Khadem AF, Pol A, Wiczorek A, Mohammadi SS, Francoijs KJ, Stunnenberg HG, Jetten**  
128 **MSM, Op den Camp HJM.** 2011. Autotrophic methanotrophy in Verrucomicrobia:  
129 *Methylacidiphilum fumariolicum* SolV uses the Calvin Benson Bassham cycle for carbon  
130 dioxide fixation. J. Bacteriol. **193**:4438-4446.
- 131 10. **Nyerges G, Han SK, Stein LY.** 2010. Effects of ammonium and nitrite on growth and  
132 competitive fitness of cultivated methanotrophic bacteria. Appl. Environ. Microbiol. **76**:5648-  
133 5651.
- 134 11. **Nyerges G, Stein LY.** 2009. Ammonia co-metabolism and product inhibition vary considerably  
135 among species of methanotrophic bacteria. FEMS Microbiol. Lett. **297**:131-136.
- 136 12. **Oh JI, Bowien B.** 1998. Structural analysis of the *fds* operon encoding the NAD<sup>+</sup>-linked  
137 formate dehydrogenase of *Ralstonia eutropha*. J. Biol. Chem. **273**:26349-26360.
- 138 13. **Op den Camp HJM, Islam T, Stott MB, Harhangi HR, Hynes A, Schouten S, Jetten MSM,**  
139 **Birkeland NK, Pol A, Dunfield PF.** 2009. Environmental, genomic and taxonomic perspectives  
140 on methanotrophic Verrucomicrobia. Environ. Microbiol. Rep. **1**:293-306.
- 141 14. **Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MSM, Op den Camp HJM.** 2007.  
142 Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature **450**:874-878.
- 143 15. **Semrau JD, DiSpirito AA, Vuilleumier S.** 2011. Facultative methanotrophy: false leads, true  
144 results, and suggestions for future research. FEMS Microbiol. Lett. **323**:1-12.
- 145 16. **Stein LY, Klotz MG.** 2011. Nitrifying and denitrifying pathways of methanotrophic bacteria.  
146 Biochem. Soc. Trans. **39**:1826-1831.
- 147 17. **Vallenet D, Engelen S, Mornico D, Cruveiller S, Fleury L, Lajus A, Rouy Z, Roche D,**  
148 **Salvignol G, Scarpelli C, Médigue C.** 2009. MicroScope: a platform for microbial genome  
149 annotation and comparative genomics. Database (Oxford) Article ID:bap021  
150 (doi:10.1093/database/bap021).