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1 GENOME ANNOUNCEMENT

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

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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. H₄MPT-linked C1-transfer genes are not

72 present. The H₄Folate-linked pathway inventory includes *metF*, *fold* and *fftL* 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 C₂ 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.

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