Supplementary Information

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Isolation and characterization of three novel mesophilic verrucomicrobial methanotrophs from volcanic soil.

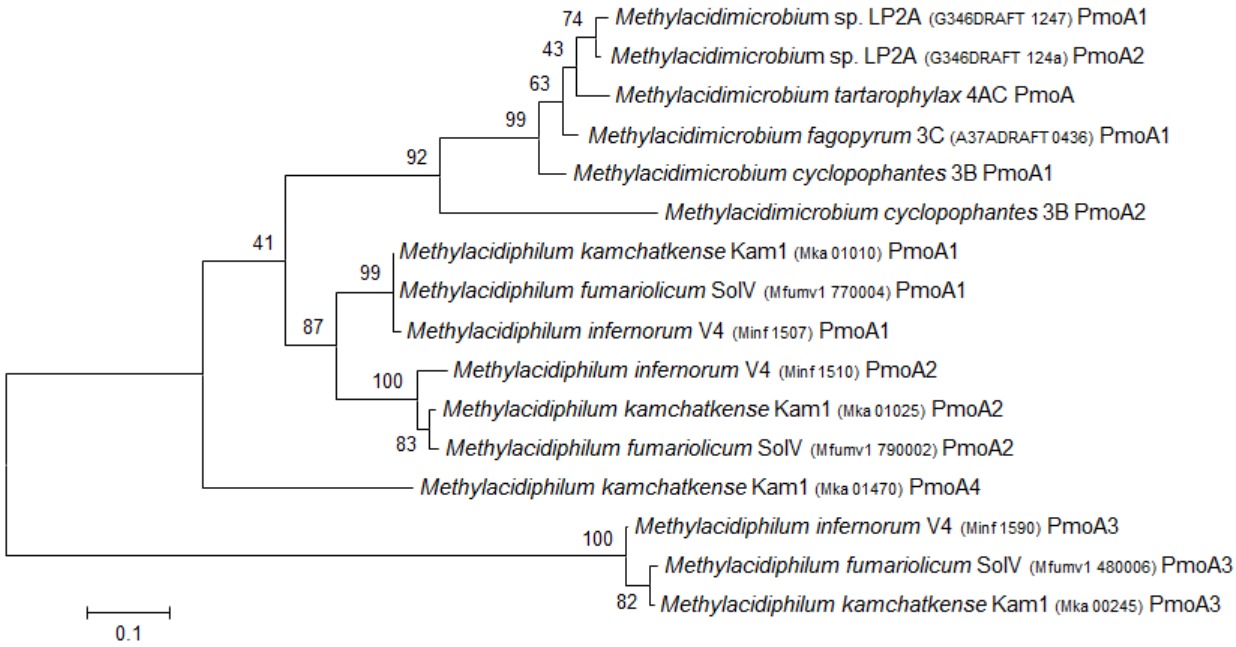

FIG S1 PmoA gene-based phylogenetic tree of methanotrophic Verrucomicrobia shows that the mesophilic strains cluster together separately from the thermophilic species. The evolutionary history was inferred by using the Maximum Likelihood method based on the Dayhoff matrix based model. The tree with the highest log likelihood (-2842.1305) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 16 amino acid sequences. All positions with less than 95% site coverage were eliminated. There were a total of 243 positions in the final dataset. Evolutionary analyses were conducted in MEGA5.

TABLE S1 Comparison based on presence/absence of key genes in methylotrophy, nitrogen metabolism and storage of polyphosphate and glycogen between the three newly described mesophilic Verrucomicrobia, strain LP2A and thermophilic species *Methylacidiphilum fumariolicum* SolV and *Methylacidiphilum infernorum* V4. Positive detection of genes was based on annotations by JGI (3C) and RAST (3B, 4AC) and on BLAST searches. In the case of strains LP2A and V4 the gene detection was noted as described previously (1). For strain SolV, the analysis was based on the previously published genome (2). + = present; ND = not detected in genome. Numbers in parentheses indicate multiple copies of a gene. * = 2nd pmoC of strain 3C is partial; ** = the NorC gene in strain 3B is fragmented.

Gene name	Product	3B	3C	4AC	LP2A	SolV	V4
Methane oxidation							
pmoC	particulate methane monooxygenase (pMMO), subunit gamma	+	+ (2)*	+	+ (3)	+ (3)	+ (4)
pmoA	particulate methane monooxygenase (pMMO), subunit beta	+ (2)	+	+ (2)	+ (2)	+ (3)	+ (3)
pmoB	particulate methane monooxygenase (pMMO), subunit alpha	+	+ (2)	+	+ (2)	+ (3)	+ (3)
mmoX	soluble methane monooxygenase (sMMO), subunit alpha	ND	ND	ND	ND	ND	ND
mmoY	soluble methane monooxygenase (sMMO), subunit beta	ND	ND	ND	ND	ND	ND
mmoZ	soluble methane monooxygenase (sMMO), subunit gamma	ND	ND	ND	ND	ND	ND
mmoB	soluble methane monooxygenase (sMMO), regulatory component	ND	ND	ND	ND	ND	ND
Methanol oxidation							
xoxF	PQQ-dependent methanol dehydrogenase	+ (2)	+ (2)	+ (2)	+ (3)	+	+
xoxJ	Periplasmic binding protein involved in methanol oxidation	+	+	+	+	+	+
xoxG	Cytochrome c family protein, electron acceptor for PQQ-methanol dehydrogenase	+	+	+	+	+	+
pqqA	coenzyme PQQ biosynthesis	+	+	ND	+	+	+
pqqB	coenzyme PQQ biosynthesis	+	+	+	+	+	+
pqqC	coenzyme PQQ biosynthesis	+	+	+	+	+	+
pqqD	coenzyme PQQ biosynthesis	+	+ (3)	+	+	+	+
pqqE	coenzyme PQQ biosynthesis	+	+	+	+	+	+
pqqF	coenzyme PQQ biosynthesis	ND	ND	ND	ND	+	ND

pqqG	coenzyme PQQ biosynthesis	ND	ND	ND	ND	ND	ND
Formate oxidation							
fdsD	NAD-dependent formate dehydrogenase subunit delta	+	+	+	+	+	+
fdsA	NAD-dependent formate dehydrogenase subunit alpha	+	+	+	+	+	+
fdsB	NAD-dependent formate dehydrogenase subunit beta	+	+	+	+	+	+
fdsG	NAD-dependent formate dehydrogenase subunit gamma	+	+	+	+	+	+
hyfB	Formate hydrogenlyase subunit 3	+ (2)	+ (2)	+ (2)	+	+	+
hyfC	Formate hydrogenlyase subunit 4	+	+	+	+	+	+
hyfE	Formate hydrogenlyase membrane component	+	+	+	+	+	+
hycG	Formate hydrogenlyase subunit 7	+	+	+	+	+	+
fdhD	Formate dehydrogenase chain D	+	+	+	+	+	+
Serine cycle (key enzyme)							
Hpr	hydroxypyruvate reductase	ND	ND	ND	ND	ND	ND
Mcl	malyl-CoA lyase	ND	ND	ND	ND	ND	ND
Ribulose monophophate pathway (key enzyme)							
hxlA	3-hexulose-6-phosphate synthase	ND	ND	ND	ND	ND	ND
hslB	6-phospho-3-hexuloisomerase	ND	ND	ND	ND	ND	ND
Calvin-Benson-Bassham Cycle							
cbbS	Ribulose 1,5 biphosphate carboxylase, small subunit	+	+	+	+	+	+
cbbL	Ribulose 1,5 biphosphate carboxylase, large subunit	+	+ (2)	+	+	+	+
Pgk	3-phosphoglycerate kinase	+	+	+	+	+	+
cbbG/gapA	Glyceraldehyde-3-phosphate dehydrogenase	+	+	+	+	+	+
tpipA	Triosephosphate isomerase	+	+	+	+	+	+
fbaA	Fructose biphosphate aldolase Class II	+	+	+	+	+	+
fbaB	Fructose-1,6-phosphate aldolase Class I (archaeal type)	+	+	+	+	+	+
Fbp	Fructose-1,6-biphosphatase	+	+	+	+	+	+
cbbT	Transketolase	+	+	+	+	+	+
Rpe	Ribulose-5-phosphate-3-epimerase	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)
xpkA	Phosphoketolase	+	+	+	+	+	+

rpiB	Ribose 5-phosphate isomerase	+	+	+	+	+	+
Udk	Phosphoribulokinase	+	+	+	+	+	+
Nitrogen metabolism							
haoAB	hydroxylamine oxidoreductase	ND	ND	ND	ND	+	+
nirK	nitrite reductase, copper-containing	+	+	+	+	+	+
nirS	cytochrome cd1 nitrite reductase	ND	ND	ND	ND	ND	ND
cytL	cytochrome P460, nitric oxide: hydroxylamine dehydrogenase	ND	ND	ND	ND	ND	ND
cytS	putative nitric oxide reductase	ND	ND	ND	ND	ND	ND
norC	nitric oxide reductase subunit C	+**	+	+	+	+	+
norB	nitric oxide reductase subunit B	ND	+	+	+	+	+
Phosphate storage genes							
ppk1	polyphosphate kinase	+	+	+	+	+	+
ppx	Exopolyphosphatase	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)	+ (2)
Glycogen genes							
glgA	glycogen synthase	+ (2)	+ (2)	+ (2)	+ (2)	+ (1)	+ (2)
gdb	glycogen debranching enzyme	+	+	+	+	+	+

TABLE S2 Dimensions of the three mesophilic verrucomicrobial methanotrophs and *M. fumariolicum* SolV averaged over 100 cells. Standard deviations are given in brackets. The width is measured at the broadest part of the cell. The length/width (L/W) ratio gives an indication of the relative width of the cell; lower values mean relatively broader cells.

	Strain 3B	**Strain 3C**	**Strain 4AC**	***M. fumariolicum***
Length (µm)	1.2 (±0.2)	1.4 (± 0.3)	1.2 (± 0.2)	1.5 (± 0.3)
Width (µm)	0.6 (± 0.1)	0.9 (± 0.1)	0.7 (± 0.1)	0.7 (± 0.1)
Ratio L/W	2.0	1.6	1.7	2.1

TABLE S3 Properties of electron dense particles observed in the four veruccomicrobial methanotrophic strains. The average diameter (standard deviation in brackets) of 24 particles measured in negatively stained cells is quite similar in the four different strains. The amount of electron dense particles per cell (again 24 particles counted) slightly differs. For each strain, the dominant elements in the ED particle as measured by EDX analysis are listed with in brackets the percentage of the 10 analyzed cells that showed this enrichment.

	Strain 3B	**Strain 3C**	**Strain 4AC**	***M. fumariolicum***
Amount of ED particles per cell	0.9	1.2	0.9	1.0
Average diameter of ED particles (µm)	0.12 (±0.04)	0.16 (±0.08)	0.15 (±0.03)	0.22 (±0.05)
ED particle is enriched in the following elements	-Phosphorus (100%) -Oxygen (80%)	-Phosporus (100%) -Oxygen (100%) -Magnesium (100%) -Nitrogen (90%)	-Sulfur (100%) -Oxygen (70%) -Phosphorus (20%)	-Oxygen (100%) -Phosphorus (70%) -Magnesium (70%) -Nitrogen (10%)

References

1. **Sharp CE, Smirnova AV, Graham JM, Stott MB, Khadka R, Moore TR, Grasby SE, Strack M, Dunfield PF.** 18 April 2014. Distribution and diversity of *Verrucomicrobia* methanotrophs in geothermal and acidic environments. Environ. Microbiol. doi:10.1111/1462-2920.12454.

2. **Khadem AF, Wieczorek AS, Pol A, Vuilleumier S, Harhangi HR, Dunfield PF, Kalyuzhnaya MG, Murrel JC, Francoijs K-J, Stunnenberg HG, Stein LY, DiSpirito AA, Semrau JD, Lajus A, Médigue C, Klotz MG, Jetten MSM, Op den Camp HJM.** 2012. Draft genome sequence of the volcano-inhibiting thermoacidophilic methanotroph *Methylacidiphilum fumariolicum* strain SolV. J. Bacteriol. **194**:3729-3730.