Methylocystis heyeri sp. nov., a novel type II methanotrophic bacterium possessing 'signature' fatty acids of type I methanotrophs

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A novel species is proposed for two strains of methanotrophic bacteria (H2^T and Sakb1) isolated from an acidic (pH 4.3) *Sphagnum* peat bog lake (Teufelssee, Germany) and an acidic (pH 4.2) tropical forest soil (Thailand), respectively. Cells of strains H2^T and Sakb1 were aerobic, Gram-negative, non-motile, straight or curved rods that were covered by large polysaccharide capsules and contained an intracytoplasmic membrane system typical of type II methanotrophs. They possessed both a particulate and a soluble methane monooxygenase and utilized the serine pathway for carbon assimilation. They were moderately acidophilic organisms capable of growth between pH 4.4 and 7.5 (optimum 5.8–6.2). The most unique characteristic of these strains was the phospholipid fatty acid profile. In addition to the signature fatty acid of type II methanotrophs (18:1 ω 8c), the cells also contained large amounts of what was previously considered to be a signature fatty acid of type I methanotrophs, 16:1 ω 8c. The DNA G + C contents of strains H2^T and Sakb1 were 61.5 and 62.1 mol%, respectively. The 16S rRNA gene sequences possessed 96–98% similarity to sequences of other type II methanotrophs in the genera *Methylosinus* and *Methylocystis*. 16S rRNA gene sequence and *pmoA* phylogeny demonstrated that the strains form a novel lineage within the genus *Methylocystis*. DNA–DNA hybridization values of strain H2^T with *Methylocystis parvus* OBBP^T and *Methylocystis echinoides* IMET 10491^T were 18 and 25%, respectively. Thus, it is proposed that these two strains represent a novel species, *Methylocystis heyeri* sp. nov. Strain H2^T (=DSM 16984^T=VKM B-2426^T) is the type strain.

Abbreviations: DMDS, dimethyldisulfide; FAME, fatty acid methyl ester; ICM, intracytoplasmic membrane; MDH, methanol dehydrogenase; PLFA, phospholipid fatty acid; pMMO, particulate methane monooxygenase; sMMO, soluble methane monooxygenase.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and partial sequences of the *mxoF*, *mmoX* and *pmoA* genes of *Methylocystis heyeri* strain H2^T are AM283543–AM283546, respectively.

A supplementary figure showing mass spectra of DMDS adducts of 16:1 PLFA of strain H2^T is available in IJSEM Online.

Aerobic methanotrophic bacteria are capable of utilizing methane as the sole source of carbon and energy. They have been divided into two groups, types I and II, belonging to the *Gamma*- and *Alphaproteobacteria*, respectively. These types differ in several phenotypic, chemotaxonomic and genotypic features, including the arrangement of intracytoplasmic membranes (ICMs), the predominant phospholipid fatty acids (PLFAs) and the pathway used for carbon assimilation. Methanotrophic bacteria inhabit a wide range of natural environments of diverse temperature, salinity and

pH (Trotsenko & Khmelenina, 2002). Only two of the 13 currently recognized genera of methanotrophs, *Methylocella* and *Methylocapsa*, are acidophilic (Dedysh *et al.*, 2000, 2002). However, molecular identification of methanotroph populations in acidic *Sphagnum*-dominated peatlands (McDonald & Murrell, 1997; Dedysh *et al.*, 2001, 2003) and acidic forest soils (Radajewski *et al.*, 2002; Knief *et al.*, 2005) has suggested that uncultivated subgroups within the genus *Methylocystis* are also capable of growth under acidic conditions. In this paper, two novel *Methylocystis* strains isolated from two acidic environments, *Sphagnum* peat and forest soil, are described. A novel species is proposed for these acidophilic methanotrophs.

Strain H2^T was isolated from a sample collected in July 2001 from 10 cm below the surface of *Sphagnum* peat (pH 4.3) on the bank of the bog lake Teufelssee in north-eastern Germany. A methanotrophic enrichment culture was obtained using liquid medium M2 at pH 5.5 as described by Dedysh *et al.* (1998). To identify methanotrophs in this enrichment, a whole-cell hybridization was applied with a set of 16S rRNA-targeted oligonucleotide probes developed for differential detection of type II methanotrophs (Dedysh *et al.*, 2003). Most of the cells in the enrichment hybridized to probe Mcyst-1261, which was designed to target a peat-inhabiting subgroup of *Methylocystis* species. Attempts to isolate these methanotrophs in pure culture by plating on agar medium M2 were unsuccessful. However, replacement of agar with gellan gum (Gel-Gro; ICN Biomedicals) resulted in the growth of methanotroph colonies and allowed us to obtain strain H2^T in a pure culture. Another strain, Sakb1, was isolated using diluted nitrate mineral salts (DNMS) medium of pH 5.8 from an acidic (pH 4.2) evergreen forest soil in Thailand (Knief *et al.*, 2005).

The isolates were maintained on their respective agar media (medium M2 for strain H2^T and DNMS medium for strain Sakb1) and in liquid cultures. For growth in liquid media, 500 ml screw-cap serum bottles were used with a headspace/liquid space ratio of 4:1. After inoculation, the bottles were sealed with silicon rubber septa and methane was added aseptically using a syringe equipped with a disposable filter (0.22 µm) to achieve a 10–20% mixing ratio in the headspace. Bottles were incubated on a rotary shaker (120 r.p.m.) at 24 °C. The absence of heterotrophic satellites was checked by phase-contrast microscopy and by plating methanotrophic isolates on 10-fold diluted Luria–Bertani agar (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl) or M2 agar medium amended with 0.1% (w/v) glucose. Morphological observations, examination of thin sections, tests for utilization of different carbon and nitrogen sources and enzyme assays were performed as described previously (Dedysh *et al.*, 2000; Dunfield *et al.*, 2003). Growth of isolates in liquid culture with methane as the sole growth substrate was monitored by nephelometry at 410 nm for 2 weeks under a variety of growth conditions, including temperatures of 4–37 °C, pH values of 4.1–8.0 and NaCl concentrations of 0.01–2.00% (w/v).

For fatty acid analyses, cells of strains H2^T and Sakb1 were grown on their respective liquid mineral media under methane and harvested in the late exponential phase. Lipids were extracted from 2.6 mg (strain Sakb1) or 6 mg (strain H2^T) freeze-dried cell material using a modified Bligh and Dyer extraction procedure (Boschker *et al.*, 1998, 2001). The lipid extract was fractionated on silicic acid into different polarity classes by sequential elution with chloroform, acetone and methanol. The methanol fraction containing the PLFA was derivatized using mild-alkaline methanolysis to

Fig. 1. Phase-contrast micrographs of cells of strains H2^T (a) and Sakb1 (b) grown on solid mineral media under methane for 7 days. (c) Electron micrograph of an ultra-thin section of a cell of strain H2^T grown on methane. Bars, 10 µm (a, b); 0.5 µm (c).

yield fatty acid methyl esters (FAMES). FAME standards of C12:0 and C19:0 were used for calculating retention indices and for quantification. Identification of FAMES was based on retention time data against known standards. For identification of methanotroph-specific PLFA, type culture extracts (*Methylobacterium methanica* NCIMB^T 11130; *Methylobacterium album* NCIMB 11123^T; *Methylobacter luteus* NCIMB 11914^T; *Methylocystis parvus* NCIMB 11129^T; *Methylosinus trichosporium* NCIMB 11131^T; *Methylosinus sporium* NCIMB 11126^T) were used as references. FAME concentrations were determined using a GC-FID system (Thermo Finnigan TRACE GC) equipped with a polar capillary column (SGE, BPX-70, 50 m × 0.32 mm × 0.25 µm) using the following oven conditions: initial temperature 50 °C for 1 min, then increasing to 130 °C by 40 °C min⁻¹, then increasing to 230 °C by 3 °C min⁻¹.

To determine double bond position of monounsaturated PLFAs, dimethyldisulfide (DMDS) derivatization was performed as described by Nichols *et al.* (1986). DMDS adducts were analysed on a Finnigan TRACE GC-MS system. DMDS adducts were separated using an Rtx-5MS (60 m × 0.32 mm × 0.25 µm) capillary column with the following oven conditions: initial temperature 80 °C for 1 min, then increasing to 160 °C by 40 °C min⁻¹, then increasing to 330 °C by 4 °C min⁻¹. MS operating parameters were: electron multiplier 375 V; source temperature 200 °C; transfer line temperature 250 °C; fullscan *m/z* 33–410. MS data were acquired and processed using the Finnigan Xcalibur software.

DNA was extracted from strains H2^T and Sakb1 using an SDS-based procedure (Dedysh *et al.*, 1998). The G+C content of the DNA was determined by thermal denaturation (Owen *et al.*, 1969) using a Unicam SP1800 spectrophotometer at a heating rate of 0.5 °C min⁻¹. The DNA of *Escherichia coli* K-12 was used as the standard. DNA–DNA hybridization of strain H2^T with *Methylocystis parvus* OBBP^T and *Methylocystis echinoides* IMET 10491^T was performed as described by Lysenko *et al.* (1988). PCR-mediated amplifications of the nearly complete 16S rRNA gene and partial fragments of the following genes (expressed protein also given) were performed: *mmoX* [α -subunit of soluble methane monooxygenase (sMMO)]; *mxoA* [large subunit of methanol dehydrogenase (MDH)] and *pmoA* [β -subunit of particulate methane monooxygenase (pMMO)]. The products were sequenced as described by Heyer *et al.* (2002). Phylogenetic sequence analyses were carried out using the ARB program package (Ludwig *et al.*, 2004).

Cells of isolates H2^T and Sakb1 were Gram-negative and non-motile. Strain H2^T formed straight polymorphic rods or ovoids (Fig. 1a), whereas strain Sakb1 formed regularly curved rods (Fig. 1b). Old cultures of strain H2^T contained many misshapen and forked cells, whereas cells of strain Sakb1 attained a distinct crescent shape, almost to a closed circle. Cells were 0.8–1.2 µm wide and 1.4–4.0 µm long, did not form rosettes during any growth stage and reproduced by normal cell division. When grown on solid media, cells

produced large capsules up to 3.0–4.0 µm thick, so that each cell was separated from other cells due to the extensive capsular material (Fig. 1a, b). No exospores or cysts were observed regardless of cell age or culture conditions. On DNMS agar, strain Sakb1 formed large (up to 1–2 cm in diameter), raised, white, slimy colonies with an entire edge and a smooth surface. Strain H2^T did not develop on agar media but on solid media made with Gel-Gro, colonies were similar to those of strain Sakb1, although smaller (up to 0.5 cm in diameter). Liquid cultures of both strains displayed white turbidity. No surface pellicle or cellular aggregates were formed. Analysis of thin sections of cells grown on methane revealed a well-developed system of ICM aligned parallel to the cytoplasmic membrane (Fig. 1c). This ICM arrangement is typical of members of the *Methylosinus*/*Methylocystis* group of type II methanotrophs.

Table 1. PLFA contents of strains H2^T and Sakb1 and other known type II methanotrophic bacteria

Strains/species: 1, strain Sakb1; 2, strain H2^T; 3, *Methylosinus* and *Methylocystis* (data from Bowman *et al.*, 1993); 4, *Methylocella* and *Methylocapsa* (Dedysh *et al.*, 2002, 2004; Dunfield *et al.*, 2003). Major PLFAs are shown in bold. PLFA shorthand nomenclature is used according to Guckert *et al.* (1985). Values given are percentages of total PLFA.

Fatty acid	1	2	3	4
14:0	0.62	<0.10	0	2.6–4.1
i15:0	0.10	<0.10	0–0.9	0.1–1.2
a15:0	0	0	0–3.5	0
15:0	0.35	<0.10	0–0.7	0–0.1
16:0	6.93	1.24	0.7–5.1	3.0–7.7
10MeC16:0	0	3.46	0	0
16:1 ω 5t	0.59	2.75	0	0
16:1 ω 8c	25.10	29.03	0	0
16:1 ω 7c	11.41	3.39	0.3–14.2	4.7–11.3
16:1 ω 7t	0	0	0	0–5.8
16:1 ω 6c	0.40	0.5	0–0.3	0
i17:1 ω 7c	0.10	<0.10	0	0–0.1
17:0	0.43	<0.10	0–0.4	0–0.1
i17:0	0	0	0–0.3	0.4–2.5
cy17:0	0.43	0.28	0–1.0	0–6.5
18:0	1.02	0.72	0–5.0	0.4–7.6
cy18:0	0	0	0–6.5	0
18:1 ω 16?	0.48	0	0	0
18:1 ω 11t	0.27	<0.10	0	0
18:1 ω 9t	1.62	14.67	0	0
18:1 ω 9c	1.62	0	0–0.4	0
18:1 ω 8c	33.33	31.98	52.9–73.6	0
18:1 ω 7c	12.88	10.86	14.8–37.7	59.2–82.2
18:1 ω 7t	0	0	0–4.6	0
18:1 ω 5c	0	0	0–0.2	0–0.1
cy19:0	0	0.18	0–0.4	0
cy19:1 ω 8c	0	0	0	0–13.6
20:0	0.14	0.12	0	0

Fig. 2. Mass spectra of DMS adducts of C16:1 PLFA of strain Sakb1. (a) The total ion chromatogram. (b–f) Scans for selected ions with m/z values that correspond to the respective Δ -fragments (see Nichols *et al.*, 1986) of DMS adducts of various C16:1 PLFA.

Strains H2^T and Sakb1 grew on methane and methanol as sole carbon and energy sources. Growth factors were not required. Methanol supported growth only when used at concentrations below 1 % (v/v); the most active growth occurred at a methanol concentration of 0.1 % (v/v). No growth was observed on other C₁ or multicarbon compounds tested. Both strains utilized ammonium salts, nitrates, yeast extract, L-alanine and L-glutamine (0.05 %, w/v) as nitrogen sources, but were also capable of growth in nitrogen-free liquid media.

The isolates grew at pH 4.4–7.5, with optimum growth at pH 5.8–6.2. This optimum and range are lower than those of other members of the genera *Methylocystis* and *Methylosinus* (Bowman *et al.*, 1993). The temperature range for growth was 5–30 °C, with optimum growth at 25 °C. No growth occurred at 37 °C. The specific growth rate of strain H2^T in liquid culture under CH₄ (10 %, v/v) at optimal temperature

and pH was 0.09 h⁻¹ (equal to a doubling time of 7.7 h). Both strains grew best on diluted media. Growth inhibition of 50 % was observed in the presence of 0.5 % NaCl (w/v) and 0.8 % NaCl completely inhibited growth.

The PLFA profiles of strains H2^T and Sakb1 deviated substantially from those of other type II methanotrophs (Table 1). The major component of these profiles was 18:1 $\omega 8c$ fatty acid. This fatty acid is common and specific to the genera *Methylosinus* and *Methylocystis* (Guckert *et al.*, 1991; Bowman *et al.*, 1993), but it is lacking in cells of members of the genera *Methylocella* and *Methylocapsa* (Dedysh *et al.*, 2002, 2004; Dunfield *et al.*, 2003). However, several other PLFAs were detected that are atypical of *Methylocystis*/*Methylosinus*. One of these was C18:1 $\omega 9t$, which comprised 14 % of the total PLFA content in strain H2^T. An even more surprising component was 16:1 $\omega 8c$, which comprised 25 and 29 % of the total PLFAs in Sakb1

and H2^T, respectively. This PLFA has previously been detected only in type I methanotrophs (*Gammaproteobacteria*) and has been regarded as a specific signature lipid for this group (Hanson & Hanson, 1996). Besides 16:1 ω 8c, the novel strains also contained 16:1 ω 5t (2.75 % in H2^T), another PLFA previously found to occur only in type I methanotrophs.

As the detection of the type I 'signature' PLFA 16:1 ω 8c in type II methanotrophs was unexpected, this result was verified via DMDS derivatization. Fig. 2 and Supplementary Fig. S1 (available in IJSEM Online) show the MS chromatograms. The total ion chromatogram (Fig. 2a) shows essentially the same output as the GC-FID chromatogram. The peak at retention time 32.84 (Fig. 2a) and 32.91 (Fig. S1a) was identified as 16:1 ω 8c. In the DMDS procedure, a mass fragment *m/z* 203 (diagnostic for 16:1 ω 8c) was formed. This comprises that part of the PLFA molecule that includes the carboxylic (Δ) end. In Fig. 2c, the *m/z* 203 Δ -fragment matched the peak in the total ion chromatogram in Fig. 2a, which was identified as 16:1 ω 8c by retention time. This identification was also confirmed using the ω -fragments of the DMDS adducts. The presence of a second 'signature' lipid of type I methanotrophs, 16:1 ω 5t, was also detected by GC-FID and DMDS analyses in both strains (Fig. 2f). This lipid was not detectable in the total ion chromatogram because the abundance of the component, indicated by the intensity of the fragment, was low. The PLFA 16:1 ω 7c could not be separated completely from 16:1 ω 8c in the GC-MS run because the DMDS adducts were analysed on an apolar column. Using the polar column for GC-FID, all compounds were separated. However, a comparison of the intensities in Fig. 2a and Fig. 2d shows that the peaks at 32.91 and 32.84, respectively, comprise the majority of the C16:1 ω 8c peak.

The possession of 16:1 ω 8c fatty acid makes these strains unique among described type II methanotrophs. Interestingly, significant amounts of 16:1 ω 8c fatty acid were detected in extracts made from acidic peat (Krumholz *et al.*, 1995; Sundh *et al.*, 1995). This was interpreted to mean that type I methanotrophs were abundant. In contrast, *in situ* hybridization with methanotroph-specific oligonucleotide probes detected only relatively small populations of type I methanotrophs in acidic peat (Dedysh *et al.*, 2001, 2003). The data presented in this study provide a possible explanation for this controversy and suggest that there are severe difficulties with the use of 16:1 ω 8c as a specific biomarker for type I methanotrophs.

The metabolic patterns of strains H2^T and Sakb1 were similar to those of other members of the genera *Methylosinus* and *Methylocystis* (Table 2). As demonstrated by the production of naphthol from naphthalene, both strains possessed sMMO. High activities of hydroxypyruvate reductase and serine-glyoxylate aminotransferase indicated that strains H2^T and Sakb1 assimilate C₁ compounds via the serine pathway. Activities of Calvin cycle enzymes

Table 2. Activities of enzymes of primary and intermediate metabolism in cell extracts of strains Sakb1 and H2^T grown on methane [nmol min⁻¹ (mg protein)⁻¹]

Enzyme	Co-factor(s)	Sakb1	H2 ^T
Methanol dehydrogenase	PMS	76	61
Formaldehyde dehydrogenase	PMS	39	46
	NAD ⁺ , GSH	0	0
Formate dehydrogenase	PMS	51	59
	NAD ⁺	0	0
Hydroxypyruvate reductase	NADH	160	134
	NADPH	114	121
L-Serine-glyoxylate aminotransferase		81	98
Ribulose-1,5-bisphosphate carboxylase		0	0
3-Hexulose phosphate synthase		0	0
Glucose-6-phosphate dehydrogenase	NAD ⁺	0	0
	NADP ⁺	68	85
6-Phosphogluconate dehydrogenase	NAD ⁺	42	42
	NADP ⁺	60	50
6-Phosphofructokinase	PP _i	154	127
	ATP	0	0
Fructose-1,6-bisphosphate aldolase	NADH	107	160
KDPG aldolase*	NADH	0	0
Pyruvate kinase	ADP	114	98
Isocitrate dehydrogenase	NAD ⁺	0	0
	NADP ⁺	183	174
2-Oxoglutarate dehydrogenase	NAD ⁺	56	40
Isocitrate lyase		0	0
Malate synthase		0	0
Glutamine synthetase	ATP, Mg ²⁺	444	325
Glutamate synthase	NADH	94	119
	NADPH	151	197
Glutamate dehydrogenase	NAD(P)H	0	0

*KDPG, 2-Keto-3-deoxy-6-phosphogluconate.

(phosphoribulokinase and ribulose-bisphosphate carboxylase) or the key ribulose monophosphate cycle enzyme (hexulose phosphate synthase) were not found. The presence of 2-oxoglutarate dehydrogenase indicates that the complete tricarboxylic acid cycle operates in this bacterium. Glutamate cycle enzymes, glutamine synthetase and glutamate synthase, were used for ammonium assimilation.

Comparative sequence analysis of the 16S rRNA gene showed that strains H2^T and Sakb1 were highly similar (>99 % 16S rRNA gene sequence similarity) and belong to the phylogenetic cluster formed by the genera *Methylosinus* and *Methylocystis* within the *Alphaproteobacteria* (type II methanotrophs) (Fig. 3). The environmental clone sequences UP4, UP5, UP6 and UP7 that were retrieved from an acidic forest soil (Radajewski *et al.*, 2002) shared an overall 16S rRNA gene sequence similarity of 98 % with the novel isolates. Differences between the novel isolates and the type strains of related species were: *Methylocystis parvus* OBBP^T (2.6–3.1 %), *Methylocystis echinoides* IMET 10491^T

Fig. 3. Phylogenetic tree based on 16S rRNA gene sequences showing the positions of strains H2^T and Sakb1 relative to type II methanotroph type strains and to other selected *Alphaproteobacteria*. Four type I methanotrophs (*Gammaproteobacteria*) were used as an outgroup. The tree was constructed by neighbour-joining (5000 data resamplings) with a Felsenstein correction. Bar, 0.1 substitutions per nucleotide position. Bootstrap values (5000 data resamplings) are shown within the *Methylocystis*/*Methylosinus* group only.

(2.6–3.1 %), *Methylocystis rosea* SV97^T (2.4–3.1 %), *Methylosinus trichosporium* NCIMB 11131^T (3.5–3.6 %) and *Methylosinus sporium* NCIMB 11126^T (3.4–3.5 %). Isolates H2^T and Sakb1 were generally more similar to species of the genus *Methylocystis* than to those of the genus *Methylosinus*. However, if all available sequences of different strains described by Heyer *et al.* (2002) are included in treeing analyses, 16S rRNA gene sequence phylogeny is variable depending on the algorithms used and the two genera are not often distinguished. Phylogenetic analysis based on partial *pmoA* sequences is better for revealing the species and genus relationships of type II methanotrophs (Heyer *et al.*, 2002). The *pmoA* phylogeny clearly showed that the novel strains represent a lineage that is distinct from all representatives of both genera, but closer to *Methylocystis* (Fig. 4).

The typical type II ICM arrangement, the pathway used for carbon assimilation, dominance of 18:1 ω 8c in PLFA profiles and results of phylogenetic analyses place strains H2^T and Sakb1 within the type II methanotroph group. Cell morphology, the absence of exospores and rosettes and the phylogeny of *pmoA* identify the novel strains as members of the genus *Methylocystis*. However, compared with recognized representatives of the genus *Methylocystis*, the novel strains differ in cell shape and by the formation of large exopolysaccharide capsules, in their preference for moderately acidic conditions and in their unique PLFA profile. The DNA–DNA hybridization values of strain H2^T with *Methylocystis parvus* OBBP^T and *Methylocystis echinoides* IMET 10491^T were 18 and 25 %, respectively. Thus, it is concluded that strains H2^T and Sakb1 represent a novel

species of the genus *Methylocystis*, for which the name *Methylocystis heyeri* sp. nov. is proposed.

Emended description of the genus *Methylocystis* (ex Whittenbury *et al.* 1970) Bowman *et al.* 1993

Methylocystis [Me.thy.lo.cys'tis. N.Gr. n. *methyl* (from Gr. n. *methu* wine and Gr. n. *hulê* wood) methyl group; N.L. fem. n. *cystis* (from Gr. fem. n. *kustis* the bladder and in biology, a cyst; N.L. fem. n. *Methylocystis* methyl cyst].

Gram-negative cells that are reniform, coccobacillary or rod-shaped; 0.3–1.2 μ m wide by 0.5–4.0 μ m long. Reproduces by normal cell division. Does not form either rosettes or exospores. May form a lipid cyst. Non-motile. Encapsulated. May accumulate poly- β -hydroxybutyrate and polyphosphate. May form spinae on cell surfaces. Contains type II ICMs that are aligned parallel to the cell wall. Possesses pMMO; some strains may possess sMMO. Grows at temperatures between 5 and 40 °C and at pH values between 4.5 and 9.0. Organic growth factors and NaCl are not required for growth. Aerobic chemolithotroph. Obligate utilizer of C₁ compounds via the serine pathway. No growth occurs on complex organic media. Does not contain the Benson–Calvin cycle for CO₂ fixation, but contains a complete tricarboxylic acid cycle. Capable of dinitrogen fixation. Produces oxidase and catalase. All representatives possess 18:1 ω 8c as the predominant PLFA; some species may also possess 16:1 ω 8c as a major PLFA. The major quinone is ubiquinone 8. The DNA G + C content ranges from 61.5 to 67.0 mol%. Phylogenetically belongs to the

Fig. 4. Phylogenetic tree constructed based on nucleotide sequences of partial *pmoA* genes, showing the relationship of strains H2^T and Sakb1 to other type I and type II methanotrophs. Some clusters are grouped for simplicity. The tree was constructed by neighbour-joining (5000 data resamplings) with a Felsenstein correction. Bootstrap values >50% are shown. A maximum-likelihood algorithm gave the same tree topology. Different strains of *Methylocystis* and *Methylosinus* used in tree construction are as described by Heyer *et al.* (2002). Bar, 0.1 substitutions per nucleotide position.

Alphaproteobacteria and is closely related to the genus *Methylosinus*. *Methylocystis parvus* is the type species.

Description of *Methylocystis heyeri* sp. nov.

Methylocystis heyeri (hey.e'ri. N.L. gen. n. *heyeri* of Heyer, named in honour of the German microbiologist Jürgen Heyer, for his highly enthusiastic research on methanotrophic bacteria and his contributions to increasing our knowledge on methanotroph diversity and ecology).

Cells are large, straight or regularly curved rods or ovoids; 0.8–1.2 µm wide and 1.4–4.0 µm long. Old cultures may contain misshapen and forked cells or nearly closed crescents. Cells possess large capsules so that each cell is separated from the others by plenty of the capsular material. Cysts are not formed. Colonies are white, highly raised and slimy. Liquid cultures display homogeneous turbidity. Grows at temperatures between 5 and 30 °C (optimum at 25 °C) and at pH values between 4.4 and 7.5 (optimum at 5.8–6.2). Carbon sources include methane and methanol. Methanol is utilized at concentrations below 1% (v/v); optimal growth occurs at 0.1% (v/v) methanol. NaCl inhibits growth at concentrations above 0.5%. The predominant PLFAs are 18:1ω8c and 16:1ω8c.

The type strain, H2^T (= DSM 16984^T = VKM B-2426^T), was isolated from the acidic *Sphagnum* peat bog lake Teufelssee, north-eastern Germany. Strain Sakb1 is a reference strain, isolated from acidic tropical forest soil (Thailand). The DNA G+C contents of strains H2^T and Sakb1 are 61.5 and 62.1 mol%, respectively.

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