***Methylocystis bryophila* sp. nov., a Novel Facultatively Methanotrophic
Bacterium from Acidic *Sphagnum* Peat, and Emended Description of the
Genus *Methylocystis* (ex Whittenbury *et al.* 1970) Bowman *et al.* 1993**

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Running title: *Methylocystis bryophila* sp. nov.

A new species is proposed for two facultatively methanotrophic representatives of the genus *Methylocystis*, strains H2s^T and S284, which were isolated from an acidic (pH 4.3) *Sphagnum* peat bog lake (Teufelssee, Germany) and an acidic (pH 3.8) peat bog (European North Russia), respectively. Cells of strains H2s^T and S284 are aerobic, Gram-negative, non-motile, curved coccoids or short rods that contain an intracytoplasmic membrane system typical of type II methanotrophs. They possess both a soluble and a particulate methane monooxygenase (MMO); the latter is represented by two isozymes, pMMO and pMMO2. The preferred growth substrates are methane and methanol. In the absence of C1 substrates, however, these methanotrophs are capable of slow growth on acetate. Atmospheric nitrogen is fixed by means of an aero-tolerant nitrogenase. Strains H2s^T and S284 grow between pH 4.2 and 7.6 (optimum pH 6.0-6.5), and at temperatures between 8 and 37°C (optimum 25-30°C). The major fatty acids are C18:1 ω 8c, C18:1 ω 7c, and C16:1 ω 7c; the major quinone is Q-8. The DNA G+C content is 62.0-62.3 mol%. Strains H2s^T and S284 share identical 16S rRNA gene sequences, which displayed 96.6-97.3% similarity to sequences of other taxonomically characterized members of the genus *Methylocystis*. Therefore, strains H2s^T and S284 are classified as a novel species, for which the name *Methylocystis bryophila* sp. nov. is proposed. Strain H2s^T (=DSMZ 21852^T = VKM B-2545^T) is the type strain of *Methylocystis bryophila*.

Keywords: *Methylocystis bryophila* sp. nov., facultative methanotrophy, acetate-utilizing methanotroph.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the partial sequences of the *mmoX*, *pmoA*, *pmoA2*, and *nifH* genes of *Methylocystis bryophila* H2s^T are FN422003- FN422005 and HE798545-HE798546, respectively.

The genus *Methylocystis* is one of the five genera which were included in the first taxonomic framework of methanotrophic bacteria created by Whittenbury and colleagues (1970). This genus belongs to the class *Alphaproteobacteria*, the family *Methylocystaceae*, and encompasses strictly aerobic utilizers of C1 compounds with type II intracytoplasmic membranes (ICM) and the serine pathway of carbon assimilation (Bowman *et al.*, 1993; Bowman, 2000). At present, the genus *Methylocystis* includes five validly described species: *M. parvus*, *M. echinoides* (Bowman *et al.*, 1993), *M. rosea* (Wartiainen *et al.*, 2006), *M. hirsuta* (Lindner *et al.*, 2007), and *M. heyeri* (Dedysh *et al.*, 2007).

For a long time, representatives of the genus *Methylocystis* were considered to be obligately methanotrophic bacteria, which are incapable of growth on multicarbon substrates. Recently, however, two novel facultatively methanotrophic members of this genus, strains H2s^T and SB2, were isolated from the acidic *Sphagnum* peat bog lake Teufelssee in northeastern Germany and a spring bog in southeast Michigan (Belova *et al.*, 2011; Im *et al.*, 2011). Both isolates demonstrated a clear preference for growth on methane but were also able to grow slowly on acetate or ethanol in the absence of methane. No growth occurred on other multicarbon substrates. 16S rRNA genes of strains H2s^T and SB2 displayed only 96% sequence similarity. Apparently, these isolates represented two different and potentially novel species of the genus *Methylocystis*. Strain SB2 was most closely related to *Methylocystis rosea* SV97^T and *Methylocystis echinoides* IMET 10491^T (98% 16S rRNA gene similarity to both strains) (Im *et al.*, 2011), while strain H2s^T showed the highest 16S rRNA gene sequence similarity (99.5%) with the taxonomically uncharacterized *Methylocystis* sp. strain F10V2a, which was isolated from the surface sediment of the dystrophic peat bog lake Fuchskuhle, northeastern Germany (Heyer *et al.*, 2002). Strain F10V2a was characterized with regard to its fatty acid composition (Bodelier *et al.*, 2009) and the presence of *pmoA2* gene encoding the β -subunit of particulate methane monooxygenase that oxidizes methane at low mixing ratios (pMMO2) (Tchawa Yimga *et al.*, 2003; Baani & Liesack, 2008). This strain, however, has been lost and could not be used for the comparative analysis.

1 Lately, we obtained one additional, strain H2s^T-like methanotroph, designated strain
2 S284. It was isolated from acidic (pH 3.8) peat sampled in August 2008 at a depth of 5-10 cm of
3 the *Sphagnum* peat bog Staroselsky Moss, Central Forest Reserve, Tver region, European North
4 Russia (56°58'N, 32°30'E). Strains H2s^T and S284 shared identical 16S rRNA gene sequences
5 and displayed similar phenotypic traits. Most importantly, strain S284 was also capable of slow
6 growth on acetate. This study was initiated in order to characterize the two novel isolates of
7 facultatively methanotrophic *Methylocystis* spp. and to determine their taxonomic position.

8 Strains H2s^T and S284 were maintained on agar and liquid medium M2 containing (in
9 grams per litre distilled water) KNO₃, 0.25; KH₂PO₄, 0.1; MgSO₄ × 7H₂O, 0.05; CaCl₂ × 2H₂O,
10 0.01; and NaCl, 0.02, with the addition of 0.1% (by volume) of a trace elements stock solution
11 containing (in grams per litre) EDTA, 5; FeSO₄ × 7H₂O, 2; ZnSO₄ × 7H₂O, 0.1; MnCl₂ × 4H₂O,
12 0.03; CoCl₂ × 6H₂O, 0.2; CuCl₂ × 5H₂O, 0.1; NiCl₂ × 6H₂O, 0.02, and Na₂MoO₄, 0.03. The
13 medium pH was 5.5. For growth in liquid media, 500 ml screw-cap serum bottles were used with
14 a headspace/ liquid space ratio of 4:1. After inoculation, the bottles were sealed with silicone
15 rubber septa, and methane was added aseptically using a syringe equipped with a disposable filter
16 (0.22 µm) to achieve a 10-20% mixing ratio in the headspace. Bottles were incubated on a rotary
17 shaker (120 rpm) at 24°C. Purity tests were performed as described by Belova *et al.* (2011) and
18 included: i) examinations using phase-contrast and electron microscopy, ii) plating on complex
19 organic media, iii) DGGE analysis of 16S rRNA gene amplicons and iv) whole-cell hybridization
20 with fluorescently labeled group- and species-specific probes M450 and Mcyst-1273.

21 Morphological observations and cell size measurements were made with a Zeiss Axioplan
22 2 microscope and Axiovision 4.2 software (Carl Zeiss, Germany). For preparation of ultrathin
23 sections, cells were pre-fixed with 1.5% (wt/vol) glutaraldehyde in 0.05M cacodylate buffer (pH
24 6.5) for 1 h at 4°C and then fixed in 1% (wt/vol) OsO₄ in the same buffer for 4 h at 20°C. After
25 dehydration in an ethanol series, the samples were embedded in a Spurr epoxy resin. Thin
26 sections were cut on an LKB-4800 microtome, mounted on copper grids covered with Formvar
27 film, contrasted with 3% (wt/vol) uranyl acetate in 70% (vol/vol) ethanol for 30 min, and then

1 stained with lead citrate (Reynolds, 1963) at 20°C for 4–5 min. The preparations were examined
2 in a JEM-100C (JEOL, Japan) electron microscope at an 80 kV accelerating voltage.

3 Physiological tests were carried out in liquid medium M2 with methane. Growth of strains
4 was monitored by measuring OD₄₁₀ for 2 weeks under a variety of conditions, including
5 temperatures of 2–37°C, pH 3.9–8.0 and NaCl concentrations of 0–3.0 % (wt/vol). The following
6 carbon sources (each at a concentration of 0.05%, w/v) were examined to determine the range of
7 substrates that could be utilized by strains H2s^T and S284: ethanol, formate, formaldehyde,
8 glucose, fructose, arabinose, lactose, sucrose, maltose, galactose, acetate, citrate, oxalate, malate,
9 pyruvate and succinate. The capacity to utilize methanol at concentrations from 0.01 to 2%
10 (vol/vol) was determined in liquid medium M2 supplemented with CH₃OH. Nitrogen sources
11 were tested by replacing KNO₃ in medium M2 with 0.05% (wt/vol) (NH₄)₂SO₄, NaNO₂, glycine,
12 L-alanine, L-serine, L-threonine, L-valine, L-leucine, L-isoleucine, L-proline, L-cysteine, L-
13 methionine, L-glutamine, L-asparagine, L-histidine, L-lysine, L-ornithine or yeast extract. In all
14 substrate utilization tests, growth was examined after 1 month of incubation and confirmed by
15 comparison with the respective negative control.

16 Cell biomass for fatty acid and isoprenoid quinone analyses as well as for DNA extraction
17 was obtained from batch cultures grown in liquid medium M2 at 24°C up to the late exponential
18 phase (7 days). Fatty acid analysis was performed as described for *Methylocystis heyeri* (Dedysch
19 *et al.*, 2007). Since fatty acid composition was not reported for *Methylocystis hirsuta* in the
20 original description (Lindner *et al.*, 2007), we determined it in our study. Isoprenoid quinones
21 were extracted according to Collins (1985) and analyzed using tandem-type mass spectrometer
22 LCQ ADVANTAGE MAX and ionization mass spectrometer Finnigan Mat 8430. The DNA
23 base composition of strains H2s^T and S284 was determined by thermal denaturation (Owen *et al.*,
24 1969) using a Unicam SP1800 spectrophotometer (UK) at a heating rate of 0.5 °C min⁻¹. The
25 DNA of *Escherichia coli* K-12 was used as a standard. PCR-mediated amplifications and
26 sequencing of the nearly-complete 16S rRNA gene and partial fragments of the *pmoA* and *mmoX*
27 genes from DNA of strain S284 were performed as described for strain H2s^T (Belova *et al.*,

2011). In addition, partial fragments of *nifH* gene from DNA of both strains were amplified and sequenced using the primers and the reaction conditions described by Dedysh *et al.* (2004). Phylogenetic analysis was carried out using the ARB program package (Ludwig *et al.*, 2004). The trees were constructed using distance-based (neighbor-joining), maximum-likelihood (DNAmI), and maximum-parsimony methods. The significance levels of interior branch points obtained in neighbor-joining analysis were determined by bootstrap analysis (1000 data re-samplings) using PHYLIP (Felsenstein, 1989).

Strains H2s^T and S284 were represented by Gram-negative, non-motile, curved coccoids or short rods (Fig. 1A). Cells were 0.9-1.4 µm wide by 1.8-3.4 µm long, occurred singly, did not form rosettes and reproduced by normal cell division. Analysis of thin sections of cells grown on methane revealed a well-developed system of intracytoplasmic membranes (ICM) aligned parallel to the cytoplasmic membrane (Fig. 1B). This ICM arrangement is typical of members of the *Methylosinus/ Methylocystis* group (Whittenbury *et al.*, 1970; Bowman, 2000). Some cells in 2-3-old batch cultures contained granules of poly-β-hydroxybutyrate. On solid M2 medium, strains H2s^T and S284 formed small (1-3 mm in diameter), raised, white or cream colonies with an entire edge and a smooth surface. Liquid cultures of both strains displayed white turbidity.

Methane was the preferred growth substrate of strains H2s^T and S284. The specific growth rate of these bacteria in liquid culture with CH₄ (10%, vol/vol) under optimal growth conditions (see below) was 0.06 h⁻¹ (equivalent to a doubling time of 11.5 h). Maximal OD₄₁₀ of methane-grown cultures of strains H2s^T and S284 ranged from 0.9 to 1.2. Methanol supported growth only at concentrations below 0.15% (vol/vol). Growth factors were not required. No growth occurred on formate, formaldehyde, glucose, fructose, arabinose, lactose, maltose, raffinose, sorbitol, mannitol, inositol, citrate, oxalate, malate, succinate, galacturonate, alanine, asparagine, glutamine, serine, glycine, pectin, or xylan. Nearly undetectable, trace growth (up to OD₄₁₀ of 0.05-0.06 after 1.5 months of incubation) occurred on pyruvate and ethanol. However, clearly detectable albeit slow growth with the specific growth rate of 0.006-0.007 h⁻¹ (equivalent to a doubling time of 100-115 h) was observed on acetate (see Belova *et al.*, 2011). The OD₄₁₀ of

acetate-grown cultures reached 0.25-0.35 following 3-4 weeks of incubation (comparing to OD₄₁₀ 0.02 in control). Both strains were able to survive multiple (4-5) transfers on a medium containing acetate. The pathway by which acetate is assimilated by these methanotrophs is still unclear, but it has been hypothesized that facultative *Methylocystis* species may utilize the coupled ethylmalonyl-CoA/serine cycle when grown on acetate (Semrau *et al.*, 2011).

Strains H2s^T and S284 utilized ammonium salts, nitrates, yeast extract, L-asparagine, L-alanine, L-serine, L-proline, L-isoleucine, L-glutamine and L-cysteine (0.05% w/v) as nitrogen sources (OD₄₁₀ of 0.2-1.0), but were also capable of weak growth in nitrogen-free liquid media (OD₄₁₀ up to 0.15 after 2 weeks of incubation). Partial *nifH* gene sequences determined for strains H2s^T and S284 (accession numbers HE798545 and HE798549) were highly similar (98.8%) to each other and displayed highest (91-92.0%) nucleotide sequence identity to the corresponding gene fragments from members of the genera *Bradyrhizobium* and *Methylocystis*.

Strain H2s^T grew in the pH range 4.4-7.6, while strain S284 was slightly more acidotolerant (pH range 4.2-7.6). Both isolates showed optimal growth at pH 6.0-6.5. This optimum and range are similar to those reported for another peat-inhabiting member of the genus *Methylocystis*, *M. heyeri* (Dedysh *et al.*, 2007). The temperature range for growth was 8-37°C, with the optimum at 25-30°C. Both strains were highly sensitive to NaCl. Growth of strain H2s^T was inhibited in the presence of 0.4% NaCl (w/v), while 0.2% NaCl completely inhibited growth of strain S284.

The major component of the PLFA (polar lipid derived fatty acids) profile in cells of strains H2s^T and S284 was C18:1 ω 8c fatty acid (Table 1). This fatty acid is commonly present in all currently known members of the genus *Methylocystis* (Bodelier *et al.*, 2009). This PLFA is also present in representatives of the genus *Methylosinus*. What distinguishes members of the genus *Methylocystis* from all other currently described methanotrophs is the presence of the PLFA's C18:2 ω 7c,12c and/or C18:2 ω 6c,12c. Among the described *Methylocystis* species, the absence of C18:2 ω 6c,12c in strains H2s^T and S284 differentiates them from *M. echnoides*, *M. parvus* and *M. hirsuta*. *M. heyeri* and *M. rosea* deviate from all other *Methylocystis* species by

the complete absence of C18:2 ω 7c,12c and or C18:2 ω 6c,12c. Another PLFA feature that distinguishes strains H2s^T and S284 from other *Methylocystis* species is the high amount of C16:1 ω 7c and C16:0.

Comparative sequence analysis of the 16S rRNA gene showed that strains H2s^T and S284 belong to the phylogenetic cluster formed by the genera *Methylocystis* and *Methylosinus* within the *Alphaproteobacteria* (type II methanotrophs) (Fig. 2). The most closely related cultured representative of these bacteria is the taxonomically uncharacterized *Methylocystis* sp. strain F10V2a (99.5% sequence similarity) from the dystrophic peat bog lake Fuchskuhle (Heyer *et al.*, 2002). Five other phylogenetically related isolates (99-100% sequence similarity), strains M162, M167, M169, M175, and M212 (GenBank accession numbers JN036511-JN036514 and JN036516), were recently obtained from an acidic *Sphagnum* peat bog at the Mariapeel nature reserve, the Netherlands (Kip *et al.*, 2011). Since only partial (800-1100 bp) nucleotide sequences of 16S rRNA genes were determined for these isolates, they were not included in the tree. 16S rRNA gene sequence difference between strains H2s^T and S284 and the type strains of other described *Methylocystis* species was 2.7-3.4%. Due to the substantial phylogenetic divergence, strains H2s^T, S284 and F10V2a fall outside a detection scope of a genus-specific probe for *Methylocystis* spp., Mcyst-1432 (Dedysh *et al.*, 2003), so that an additional probe, Mcyst-1273, was designed for specific detection of these methanotrophs (Belova *et al.*, 2011).

Phylogenetic analysis based on fragments of the *pmoA1* gene, which encodes the β -subunit of pMMO, revealed that strains H2s^T, S284, and F10V2a possess identical nucleotide *pmoA1* sequences but display only 87.9-90.0% nucleotide sequence identity (or 90.7-93.4% derived amino acid sequence identity) to *pmoA1* gene fragments from other taxonomically characterized members of the genus *Methylocystis* (Fig. 3). The presence of *pmoA2* encoding the second type of pMMO (pMMO2) in *Methylocystis* sp. strain F10V2a was demonstrated before with the use of primers designed to specifically target this gene (Tchawa Yimiga *et al.*, 2003). The *pmoA2* gene sequences determined in our study for strains H2s^T and S284 were identical to that in strain F10V2a (Fig. 3). Unexpectedly, *pmoA2* fragments were amplified from DNA of strains

H2s^T and S284 using the primer combination A189f - A682b (Holmes *et al.*, 1995), which is routinely employed for *pmoA1* detection in methanotrophs. The conventional PCR carried out at the annealing temperature 50°C with this primer combination and template DNA from strain H2s^T yielded both the *pmoA1* and *pmoA2* gene fragments in relation 4:1. Further comparison of the PmoA2 sequences from the novel isolates with those in the public database revealed their high similarity (97.7-98.3%) to the inferred peptide sequences of *pmoA* clones designated P13.6, P12.12 and P12.8 (accession numbers AY080950, AY080958, AY080959), which were retrieved by means of stable isotope probing technique from an acidic forest soil incubated under ¹³CH₄ (Radajewski *et al.*, 2002). Apparently, these clones represent *pmoA2* gene fragments from strain H2s^T-like methanotrophs. Another highly similar PmoA2 sequence (accession number FR726179) was retrieved from an acidic fen in Germany (Wieczorek *et al.*, 2011). The identity values between the PmoA2 sequences of strains H2s^T, S284, and F10V2a and the PmoA2 sequences from other known alphaproteobacterial methanotrophs comprised 89.0-91.8%, respectively.

Strains H2s^T, S284, and F10V2a are, therefore, the first characterized representatives of peat-inhabiting methanotrophs that possess pMMO2. The latter is responsible for oxidizing methane at low mixing ratios, even at the trace level of atmospheric methane (Baani & Liesack, 2008). Notably, high-affinity methane oxidation has earlier been detected in a *Sphagnum*-derived peat (Dedysh & Panikov, 1997; Wieczorek *et al.*, 2011). Given that strain H2s^T-like bacteria represent one of the dominant methanotroph populations in *Sphagnum*-dominated northern wetlands (Belova *et al.*, 2011; Kip *et al.*, 2011), their contribution to the processes of atmospheric methane consumption might have been underestimated.

The partial nucleotide sequences of the *mmoX* gene, encoding the α -subunit of sMMO hydroxylase, were also nearly identical in strains H2s^T, S284 and *Methylocystis* sp. strain F10V2a, but displayed only 85.7-88.3% nucleotide sequence identity (or 93.6-95.7% derived amino acid sequence identity) to *mmoX* gene fragments from other members of the *Methylosinus*/*Methylocystis* group (Fig. 4). As shown in our previous study (Belova *et al.*, 2011),

mmoX was not expressed in strain H2s^T during its growth either on methane or acetate in Cu-containing medium.

In summary, the 16S rRNA, *pmoA* and *mmoX* gene phylogenies demonstrated that strains H2s^T and S284 form a novel lineage within the genus *Methylocystis*. The ability to grow in acidic conditions and to utilize acetate differentiated the two novel strains from other currently characterized *Methylocystis* species, with the only exception of *M. heyeri* (Table 2). The latter could be distinguished from strains H2s^T and S284 by the presence of large polysaccharide capsules and the occurrence of C16:1 ω 8c in the PLFA profile. High amounts of C16:0 and C16:1 ω 7c and the absence of C18:2 ω 6c,12c differentiated the two novel strains from *M. echinoides*, *M. parvus* and *M. hirsuta*. Absence of pigments made strains H2s^T and S284 different from *M. rosea*, while the presence of sMMO differentiated them from *M. rosea* and *M. echinoides*. These phenotypic, metabolic and genotypic differences between strains H2s^T and S284 and other taxonomically characterized members of the *Methylocystis* suggest that it represents a novel species within this genus, for which we propose the name *Methylocystis bryophila* sp. nov.

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

Methylocystis (Me.thyl.o.cys'tis. N.L. n. *methylum*, methyl group; L. f. *cystis*, a cyst; N.L. f.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. Nonmotile. Encapsulated. May accumulate poly- β -hydroxybutyrate and polyphosphate. May form spinae on cell surfaces. Contains type II intracytoplasmic membranes which are aligned parallel to the cell wall. Possess 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. Utilizes C₁

compounds via the serine pathway. May utilize acetate or ethanol. 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. In case of methanotrophic bacteria the PLFA's C18:2 ω 7c,12c and or C18:2 ω 6c,12c have only been demonstrated to be present in the genus *Methylocystis* and can thus be regarded as a diagnostic lipid for this genus. The major quinone is ubiquinone 8. The DNA base composition ranges from 61.5 to 67.0 mol%. Phylogenetically belongs to the *Alphaproteobacteria* and is closely related to the genus *Methylosinus*. *Methylocystis parvus* is the type species.

Description of *Methylocystis bryophila* sp. nov.

Methylocystis bryophila (bry.o'phi.la. Gr. neut. n. *bryon* moss; Gr. adj. *philus* loving; N.L. fem adj. *bryophila* moss-loving intended to mean moss-associated).

Gram-negative, curved coccoids, 0.9-1.4 μ m wide by 1.8-3.4 μ m long. Cells occur singly, do not form rosettes. Reproduce by binary fission. Non-motile. Produce intracellular PHB granules. Cells contain a well developed, type II ICM system. Possess both pMMO and sMMO. The temperature range for growth is 8-37°C with the optimum at 25-30°C. Growth occurs between pH 4.2 and 7.6 with the optimum at pH 6.0-6.5. The preferred growth substrate is methane. Methanol is utilized at concentrations below 0.1% (vol/vol). Growth factors are not required. Capable of slow growth on acetate. Nitrogen sources are ammonium salts, nitrates, yeast extract, L-asparagine, L-alanine, L-serine, L-proline, L-isoleucine, L-glutamine and L-cysteine. Capable of N₂ fixation. NaCl inhibits growth at a concentration above 0.1% (wt/vol). The major PLFAs are C18:1 ω 8c, C18:1 ω 7c and C16:1 ω 7c. The major quinone is Q-8. The DNA G+C content is 62.0-62.3 mol%. Habitats are acidic wetlands. The type strain is strain H2s^T (= DSMZ 21852^T = VKM B-2545^T), which was isolated from the acidic peat bog lake Teufelssee, Northeastern Germany.

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- 8

- 1 **Table 1.** PLFA contents of strains H2s^T and S284 in comparison to other species of the genus
- 2 *Methylocystis*. Major fatty acids are shown in bold.

Fatty acid	<i>Methylocystis bryophila</i>			<i>Methylocystis heyeri</i> ^c	<i>M. rosea</i> ^d	<i>M. echi-noides</i> ^b	<i>M. parvus</i> ^a	<i>M. hirsuta</i> ^a
	H2s ^{Ta}	S284 ^a	F1V2a ^b					
C14:0	-	-	0.1	0.6	-	-	-	-
iC15:0	0.2	0.2	0.3	0.1	-	-	-	-
aC15:0	-	-	-	-	-	-	-	-
C15:0	-	0.1	0.2	0.4	-	-	-	-
10MeC16:0	-	-	-	3.5	-	-	-	-
C16:1ω8c	-	-	-	25.1-29.3	-	-	-	-
C16:1ω7c	15.4	16.7	12.4	3.4-11.4	6.1	0.8	0.5	0.3
C16:1ω6c	-	-	0.1	0.4-0.5	-	-	-	-
C16:1ω5t	-	-	-	0.6-2.8	-	-	-	-
C16:0	2.4	2.4	2.7	1.2-6.9	-	0.3	0.2	0.2
iC17: 1ω7c	-	-	-	0.1	-	-	-	-
iC17:0	0.2	-	-	-	-	-	-	-
C17:1ω8c	0.2	0.2	-	-	-	-	-	-
C17:0	-	-	-	0.4	-	-	-	-
cyC17:0	-	-	0.2	0.3-0.4	-	-	-	-
C18:1ω9t	-	-	0.7	1.6-14.7	-	1.5	-	-
C18:1ω8t	0.8	1.6	-	-	-	-	-	1.5
C18:1ω9c	-	-	-	1.6	-	-	-	-
C18:1ω8c	53.1	52.8	33.6	32.0-33.3	54.2	51.9	45.5	57.2
C18:1ω7c	19.3	18.4	45.1	1.9-12.9	39.7	18.1	23.7	15.4
C18:1ω6c	-	-	-	-	-	-	-	0.1
C18:1ω5c	-	-	-	-	-	0.3	-	-
C18:0	-	-	0.2	0.7-1.2	-	0.2	-	0.2
C18:2ω7c, 12c	7.8	7.0	4.5	-	-	22.7	8.0	20.3
C18:2?	-	0.2	-	-	-	-	-	0.2
C18:2ω6c, 12c	-	-	-	-	-	4.4	22.1	4.4
brC19:0	-	-	-	-	-	-	-	-
cyC19:0	-	-	-	0.2	-	-	-	-
C20:0	-	-	-	0.1	-	-	-	-

- 3 ^a – data obtained in this study, ^b – data from Bodelier *et al.* (2009), ^c – data from Dedysh *et al.*
- 4 (2007), d- data from Wartainen *et al.* (2006), ^e – data from Bowman *et al.* (1991).

1 **Table 2.** Major characteristics that distinguish *Methylocystis bryophila* sp. nov. from other described species of the genus *Methylocystis*: 1 –
2 *Methylocystis bryophila* sp. nov., 2 – *M. heyeri* (data were taken from Dedysh *et al.* (2007)), 3 – *M. rosea* (data were taken from Warttiainen *et al.*
3 (2006)), 4 – *M. echinoides* (data were taken from Gal'chenko *et al.* (1977), Bowman *et al.*(1993)), 5 – *M. parvus* (data were taken from Bowman *et al.*
4 (1993)), 6 – *M. hirsuta* CSC1^T (data were taken from Lindner *et al.* (2007))

Characteristic	1	2	3	4	5	6
Cell shape	Small curved coccoids, short rods	Straight polymorphic or regularly curved rods, ovoids	Rods	Coccobacilli, rods	Rods, coccobacilli	Dumbbell
Cell size (µm)	0.9-1.4 × 1.8-3.4	0.8-1.2 × 1.4-4.0	0.8-1.1 × 1.1-2.5	0.6 × 0.8-1.2	0.5-0.8 × 1.0-2.0	0.3-0.6 × 0.7-1.0
Color of colonies	Light cream	White	Pink-red	White, buff, pale pink	Diffusible brown, pale pink	Cream
Optimal growth temperature (°C)	25-30	25	27	27-30	28-30	30
Temperature range (°C)	8-37	5-30	5-37	15-30	15-37	ND
pH range	4.2-7.5	4.4-7.5	5.0-9.0	5.5-8.5	5.0-9.0	ND
Optimal growth pH	6.0-6.5	5.8-6.2		6.5-7.5	6.5-7.5	7.0
Growth on methanol (%)	≤0.15	≤1	-	0.2	0.1-0.3	+
Growth on acetate*	+	w	-	w	-	w
sMMO	+	+	-	-	-	+
Major quinone(s)	Q-8	Q-8	ND	Q-8	Q-8	Q-8
PLFA C18:2ω7c, 12c	+	-	-	+	+	+
DNA G + C content (mol%)	62.3	61.5-62.1	62	62	64-67	ND

5 ND, not determined; w – weakly positive.

6 * - data were taken from Belova *et al.* (2011).

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

FIGURE CAPTIONS

Fig. 1. (A) Phase-contrast micrograph of cells of strains H2s^T grown in liquid mineral medium M2 under methane for 7 days; bar, 5 μ m. (B) Electron micrograph of ultrathin section of a cell of strain H2s^T; bar, 0.5 μ m.

Fig. 2. 16S rRNA gene-based neighbor-joining tree showing the phylogenetic positions of strains H2s^T, S284 and F10V-2a in relation to other taxonomically characterized members of the family *Methylocystaceae* and phylogenetically related representatives of the family *Beijerinckiaceae*. Bootstrap values (percentages of 1000 data resamplings) >50% are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The type I methanotrophs *Methylobacterium album* (X72777), *Methylobacter luteus* (AF304195), *Methylomonas methanica* S1 (AF304196) and *Methylococcus capsulatus* Texas (AJ563935) were used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

Fig. 3. Neighbor-joining tree constructed based on 150 deduced amino acid sites of partial *pmoA1* and *pmoA2* gene sequences, showing the positions of strains H2s^T, S284 and F10V2a relative to other type II methanotrophs. Bootstrap values (1000 data resamplings) >80% are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. The type I methanotrophs *Methylomonas methanica* (U31653), *Methylobacter psychrophilus* (AY945762), *Methylobacterium album* (U31654), *Methylocaldum tepidum* (U89304), *Methylocaldum szegediense* (U89303), and *Methylococcus capsulatus* (L40804) were used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

Fig. 4. Unrooted neighbor-joining tree constructed based on 328 deduced amino acid sites of partial *mmoX* gene sequences, showing the positions of strains H2s^T and S284 relative to other sMMO-possessing type I and type II methanotrophs. Bootstrap values (1000 data resamplings)

>80% are shown. Black circles indicate that the corresponding nodes were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.05 substitutions per nucleotide position.