Methylobacterium cerastii sp. nov., isolated from the leaf surface of *Cerastium holosteoides*

S. Wellner, N. Lodders and P. Kämpfer

Correspondence

S. Wellner
stefanie.a.wellner@umwelt.uni-
giessen.de

Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

Two Gram-stain-negative, non-endospore-forming, rod-like strains, designated C15^T and C44, were isolated from the phyllosphere of *Cerastium holosteoides* and were studied in detail in order to assess their taxonomic position. 16S rRNA gene sequence analysis allocated both isolates clearly to the genus *Methylobacterium*. Both strains showed the highest 16S rRNA gene sequence similarity to *Methylobacterium marchantiae* JT1^T (97.5%) and *Methylobacterium jeotgali* S2R03-9^T (97.4%). The fatty acid profiles contained major amounts of C_{16:0}, C_{18:1ω7c} and C_{16:1ω7c/iso-C_{15:0}} 2-OH (summed feature 3), which supported the grouping of the isolates in the genus *Methylobacterium*. Physiological/biochemical characterization and DNA–DNA hybridizations with the type strains of the most closely related species allowed a clear phenotypic and genotypic differentiation of the strains. For this reason, we propose for strain C15^T (=DSM 23679^T =CCUG 60040^T =CCM 7788^T) a novel species with the name *Methylobacterium cerastii* sp. nov. Strain C44 (=DSM 23675 =CCM 7789) is an additional strain of *M. cerastii*.

The genus *Methylobacterium* was initially proposed by Patt *et al.* (1976) with the type species *Methylobacterium organophilum* and is classified in the class Alphaproteobacteria. Species of the genus *Methylobacterium* are strictly aerobic, facultatively methylotrophic, Gram-negative, rod-shaped bacteria that can grow on single-carbon compounds such as formate, formaldehyde and methanol as the sole source of carbon and energy, as well as on a wide range of multicarbon growth substrates (Green, 2006).

Methylobacterium strains are sometimes called pink-pigmented facultative methylotrophs (PPFMs) due to their characteristic pink pigmentation. These pigments are carotenoids, mainly xanthophylls (Urakami *et al.*, 1993; Konovalova *et al.*, 2007). However, it should be noted that two type strains of the genus, *Methylobacterium nodulans* ORS 2060^T and *Methylobacterium jeotgali* S2R03-9^T, are not pigmented.

At the time of writing, the genus *Methylobacterium* comprised 35 recognized species: *M. adhaesivum* (Gallego *et al.*, 2006), *M. aerolatum* (Weon *et al.*, 2008), *M. aminovorans* (Urakami *et al.*, 1993), *M. aquaticum* (Gallego *et al.*, 2005a), *M. brachiatum* (Kato *et al.*, 2008), *M. chloromethanicum* (McDonald *et al.*, 2001), *M. dichloromethanicum* (Doronina *et al.*, 2000),

M. extorquens (Bousfield & Green, 1985), *M. fujisawaense* (Green *et al.*, 1988), *M. gregans* (Kato *et al.*, 2008), *M. hispanicum* (Gallego *et al.*, 2005a), *M. iners* (Weon *et al.*, 2008), *M. isbiliense* (Gallego *et al.*, 2005c), *M. jeotgali* (Aslam *et al.*, 2007), *M. komagatae* (Kato *et al.*, 2008), *M. lusitanum* (Doronina *et al.*, 2002), *M. marchantiae* (Schauer *et al.*, 2011), *M. mesophilicum* (Green & Bousfield, 1983), *M. nodulans* (Jourand *et al.*, 2004), *M. organophilum* (Patt *et al.*, 1976), *M. oryzae* (Madhaiyan *et al.*, 2007), *M. persicinum* (Kato *et al.*, 2008), *M. phyllosphaerae* (Madhaiyan *et al.*, 2009), *M. platani* (Kang *et al.*, 2007), *M. podarium* (Anesti *et al.*, 2004), *M. populi* (Van Aken *et al.*, 2004), *M. radiotolerans* (Green & Bousfield, 1983), *M. rhodesianum* (Green *et al.*, 1988), *M. rhodinum* (Green & Bousfield, 1983), *M. salsuginis* (Wang *et al.*, 2007), *M. suomiense* (Doronina *et al.*, 2002), *M. tardum* (Kato *et al.*, 2008), *M. thiocyanatum* (Wood *et al.*, 1998), *M. variabile* (Gallego *et al.*, 2005b) and *M. zatmanii* (Green *et al.*, 1988). In addition, the species '*Methylobacterium goesingense*' was proposed by Idris *et al.* (2006) but the name has not been validly published.

Members of the genus *Methylobacterium* are ubiquitous in nature and can be found in such diverse habitats as soil, freshwater, sewage, in the human mouth and on feet (Doronina *et al.*, 2002; Kato *et al.*, 2008; Anesti *et al.*, 2004; Anesti *et al.*, 2005). They are known particularly for their close association with plants (Corpe & Rheem 1989; Holland & Polacco, 1994; Lidstrom & Chistoserdova, 2002; Sy *et al.*, 2005). They have been isolated for example from stem tissue of rice (Madhaiyan *et al.*, 2007), leaf tissue of rice (Madhaiyan *et al.*, 2009) and leaf samples of plane

Abbreviation: pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains C15^T and C44 are FR733885 and FR733886, respectively. The accession numbers for the *mxoF* gene sequences of strains C15^T and C44 are FR847841 and FR847842, respectively.

A supplementary figure is available with the online version of this paper.

Table 1. Differential phenotypic characteristics of the novel isolates and related species of the genus *Methylobacterium*.

Taxa: 1, strain C15^T; 2, strain C44; 3, *M. organophilum* LMG 6083^T; 4, *M. jeotgali* S2R03-9^T; 5, *M. phyllosphaerae* DSM 19779^T; 6, *M. oryzae* DSM 18207^T; 7, *M. fujisawaense* DSM 5686^T; 8, *M. marchantiae* JT1^T. +, Positive; –, negative; (+), weak; v, variable results. Data were obtained comparatively in this study. Results in brackets are from: 3, Patt *et al.* (1976, 1974); 4, Aslam *et al.* (2007); 5, Madhaiyan *et al.* (2009); 6, Madhaiyan *et al.* (2007); 7, Green *et al.* (1988); 8, Schauer *et al.* (2011). All strains were positive for hydrolysis of bis-*p*-nitrophenyl (bis-*p*NP) phosphate* and L-alanine *p*-nitroanilide (pNA)*† and negative for hydrolysis of *p*NP β-D-xylopyranoside*. All strains were negative for acid production from D-trehalose* and assimilation of maltitol*, D-mannitol* and L-leucine*.

Characteristic	1	2	3	4	5	6	7	8
Acid produced from:								
D-Glucose*/‡	–/–	–/–	+/–	–/–	–/–	–/–	–/–	–/–
L-Arabinose*	–	+	+	–	+	+	+	–
D-Xylose*/‡	–/+	–/+	+/+	–/+	+/+	+/+	+/+	–/+
D-Mannose*	–	–	+	–	–	–	–	–
Rhamnose‡	+	+	+	+	+	+	+	+
Assimilation as sole carbon source								
Methane§	–	–	– [+]	– [–]	[–]	[–]	[–]	–
Formamidell	+	–	–	+	+	–	(+)	+
Formaldehydell	–	–	–	(+) [+]	[–]	[–]	–	–
Methylaminell	–	–	– [–]	[+]	[(+)]	[–]	[–]	–
Formatell	–	–	–	–	(+) [+]	[+]	–	–
L-Glutamate†	(+)	+	+	+	+	[+]	+	+
Tartrate†	–	–	–	–	– [–]	– [–]	– [V]	– [+]
Ethanol†	–	–	+	[+]	+	+	[(+)]	+
N-Acetyl D-galactosamine*	–	–	+	–	–	–	–	–
N-Acetyl D-glucosamine*	+	–	–	–	–	–	–	–
L-Arabinose*/*	–/(+)	–/+	–/–	–/–	–/+ [+]	+/+ [+]	+/+ [+]	–/– [–]
D-Fructose*/†	–/–	–/–	+/+	–/– [–]	–/– [+]	–/– [–]	+/+ [V]	+/+ [+]
D-Galactose*	–	–	– [+]	–	+	–	–	–
D-Glucose*/†	–/–	–/–	+/+ [+]	–/– [–]	–/+ [(+)]	–/– [–]	–/+ [+]	–/– [–]
D-Ribose*	–	+	– [–]	–	–	+	+	–
Sucrose*	–	–	–	–	+	–	–	–
Trehalose*	–	–	+	–	–	–	–	–
D-Xylose*/†	–/–	–/–	+/–	–/– [+]	+/+ [+]	–/+ [–]	–/+ [+]	–/– [–]
D-Sorbitol*	–	+	–	–	–	–	–	–
Putrescine*	+	–	–	–	–	–	–	–
Sodium acetate*/†	–/–	–/–	+/+ [+]	+/– [+]	–/+ [(+)]	–/+ [–]	–/– [+]	+/+ [+]
Propionate*	–	–	+	+	–	–	–	+
cis-Aconitate*	–	+	–	–	–	–	–	–
trans-Aconitate*	–	+	–	–	–	–	–	–
Adipate*	–	+	–	–	+	+	+	–
Azolate*	–	+	–	–	+	+	+	–
Citrate*/†	–/–	+/–	–/–	–/– [–]	–/– [+]	–/– [–]	–/– [+]	–/– [(+)]
Fumarate*	–	+	+	+	–	–	–	+
Glutarate*	–	+	+	–	–	–	+	–
DL-3-Hydroxybutyrate*	–	+	+	+	–	+	+	+
Itaconate*	–	–	+	–	(+)	–	–	–
DL-Lactate*	–	+	+	+	+	–	–	+
L-Malate*	–	+	+	[+]	+	–	–	+
Mesaconate*	–	+	–	–	–	–	–	–
Oxoglutarate*	+	+	–	–	–	–	–	+
Pyruvate*	–	+	+	+	–	–	–	+
Suberate*	–	+	(+)	–	+	+	+	–
L-Alanine*	–	–	–	–	+	–	–	–
β-Alanine*	–	–	(+)	–	–	–	–	–
L-Aspartate*	–	–	+	+	+	–	–	+
L-Histidine*	–	+	–	–	–	–	–	–
L-Phenylalanine*	–	+	–	–	–	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8
L-Tryptophan*	–	–	+	–	–	–	–	–
3-Hydroxybenzoate*	–	+	–	–	–	–	–	–
DL-3-Phenylacetate*	–	+	–	–	–	–	–	–
Hydrolysis of:								
pNP β -D-glucopyranoside*	–	+	–	–	–	–	–	–
pNP phenylphosphonate*	+	+	–	–	+	+	+	+
2-Deoxythymidine-5-pNP phosphate*	+	+	+	–	+	+	+	–
L-Glutamate- γ -carboxy pNA*	–	–	–	+	–	–	–	–

*Result from F-panel according to Kämpfer *et al.* (1991).

†Growth in M125 supplemented with 0.5 % substrate.

‡Result from Micronaut E.

§Growth in M125 where methane was provided as head space gas in the proportion 50:50 methane/air.

||Growth in M125 supplemented with 0.1 % substrate.

(Kang *et al.*, 2007), internal poplar tissue (Van Aken *et al.*, 2004) and root nodules (Jourand *et al.*, 2004). Associations of members of the genus *Methylobacterium* with plants range from epiphytic to endophytic and symbiotic relations (Sy *et al.*, 2001; Koenig *et al.*, 2002; Pirttilä *et al.*, 2000; Idris *et al.*, 2006). A recent culture-independent analysis showed that the genus *Methylobacterium* is one of the predominant genera in the phyllosphere, together with the genera *Sphingomonas* and *Pseudomonas* (Delmotte *et al.*, 2009). Methylobacteria are able to utilize the methanol emitted from plants and, in turn, produce plant-growth-promoting substances such as indole-3-acetic acid (IAA), cytokinins or vitamins (Ivanova *et al.*, 2000; Ivanova *et al.*, 2001; Ivanova *et al.*, 2006; Koenig *et al.*, 2002; Trotsenko *et al.*, 2001).

Strains C15^T and C44 were isolated from the leaf surface of *Cerastium holsteoides* plants, collected in the Hainich-Dün region, Germany, a region that is systematically studied in the framework of a comprehensive biodiversity program (Fischer *et al.*, 2010).

Bacteria were removed from the leaf surface with potassium phosphate buffer (6.75 g KH₂PO₄, 8.75 g K₂HPO₄ per litre) and mechanical treatment (Stomacher 80 Biomaster; Seward Laboratory Systems). Serial dilutions were plated on mineral salt medium supplemented with 0.5 % methanol (M125, according to DSMZ) and incubated at 25 °C for 14 days. Pink-pigmented colonies with different morphology were isolated.

Cellular morphology and motility were determined microscopically (fluorescence microscope Axiophot2; Zeiss) using exponentially grown M125 broth cultures. Gram-staining was performed as described by Gerhardt *et al.* (1994). Both strains were aerobic, Gram-stain-negative, rod-shaped, non-motile and formed pink- to red-pigmented colonies. Production of the enzymes catalase and oxidase was tested positive with 3 % H₂O₂ and 1 % tetramethyl-*p*-phenylenediamine dihydrochloride, respectively, according to Gerhardt *et al.* (1994).

Growth on different media was monitored over 21 days at 28 °C. The tested media were: Caso (Carl Roth), GP (M85,

according to DSMZ), K7 (M1199, according to DSMZ), LB (Sigma-Aldrich), M65 (according to DSMZ), marine (Becton Dickinson), NA (Becton Dickinson), nutrient (Oxoid), PCA (5 g casein peptone, 2.5 g yeast extract, 1 g glucose, 9 g agar per litre, pH 7.0), PYE (3 g yeast extract, 3 g peptone, 15 g agar per litre, pH 7.2), PYG (20 g glucose, 10 g yeast extract, 10 g bacto peptone, 15 g agar per litre), R2A (Oxoid), TGE (M1207, according to DSMZ) and TS medium (Becton Dickinson).

For various physiological tests, bacteria were grown in medium M125. Growth at various temperatures (4, 10, 16, 20, 25, 28, 36 and 45 °C), different initial methanol concentrations (0.1, 0.3, 0.5, 0.7, 1.0, 1.5 and 2.0 %) and different initial pH (4.0, 5.0, 6.0, 7.0, 7.2, 8.0 and 10.0) was monitored after 3 and 14 days. Salt (NaCl)-tolerance was assessed in M125 broth supplemented with 0, 0.5, 1.0, 2.0, 3.0 and 4.0 % (w/v) NaCl over a period of 14 days. Assimilation of acetate, L-arabinose, citrate, ethanol, D-fructose, D-glucose, L-glutamate, tartrate and D-xylose was monitored in M125 broth supplemented with 0.5 % substrate. Growth on different C₁ compounds was tested in M125 broth supplemented with 0.1 % formaldehyde, formate, formamide or methylamine, respectively, instead of methanol. Assimilation of methane was carried out in M125 broth where methane was provided as head space gas in the proportions 50:50 methane/air according to Green (2006). Hydrolysis of starch was tested with iodine solution after growth on modified Bennett agar (Williams *et al.*, 1989). Indole formation, hydrolysis of adonitol, citrate, glucose, inositol, malonate, n-nitrophenyl galactopyranoside, rhamnose, sucrose and xylose as well as production of the enzymes arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease were determined with the micronaut E test plate (Merlin Diagnostika) following the manufacturer's instructions. Comparative physiological characterization of the isolates and the most closely related species of the genus *Methylobacterium* were carried out according to Kämpfer *et al.* (1991).

Like other members of the genus, both strains grew on methanol. Strain C15^T grew on formamide, but not on formaldehyde, formate or methylamine. Strain C44 did not grow on C₁ compounds other than methanol. The positive control, which consisted of M125 broth containing 0.1 % methanol, showed growth for all tested strains. Neither strain grew on methane. *M. organophilum* LMG 6083^T also did not grow on methane although it was described to utilize methane (Patt *et al.*, 1974; Patt *et al.*, 1976). However, Green & Bousfield (1983) previously reported that *M. organophilum* lost its ability to utilize methane. Other physiological features are summarized in the species description. The strains differed from their closest relatives in several carbon-source utilization features (Table 1). In contrast to *M. marchantiae* JT1^T and *M. jeotgali* S2R03-9^T, neither strain utilized L-aspartate, ethanol or propionate as sole carbon source but hydrolysed *p*-nitrophenyl (pNP) phosphate.

For the extraction of pigments, bacteria were grown in R2A broth (Lab M). Bacterial suspension was centrifuged for 5 min at 14 000 r.p.m., washed twice with 1 × PBS and dissolved in 80 % (v/v) methanol. After sonification for 7 min, the bacterial suspension was incubated at −20 °C for 24 h in the dark. The resulting suspension was centrifuged for 5 min at 14 000 r.p.m. to remove the insoluble cell debris. Thereafter, the supernatant was measured at wavelengths from 300 to 900 nm in an Infinite 200 Pro microplate reader (Tecan) against 80 % methanol as reference. Since carotenoids are known to be esterified by long chain fatty acids, an additional saponification procedure was applied to a subsample of the extracted pigments; 0.4 ml 0.01 M NaOH was added to 1 ml extract and the mixture was incubated at room temperature for 8 h in the dark (Stepnowski *et al.*, 2004). For both isolates, the pigments extracted with methanol showed absorption peaks at 490 and 520 nm, respectively, and a weak peak at 460 nm. These absorption maxima have also been reported by Schauer *et al.* (2011) and Urakami *et al.* (1993) indicating that strains C15^T and C44 contain the same carotenoids as previously described *Methylobacterium* strains. In addition, a peak at 314 nm was detected for isolate C15^T. Furthermore, a peak at 360 nm was detected in methanolic cell extracts which was also present in the non-pigmented control *M. jeotgali* S2R03-9^T. There was no absorption maximum at 770 nm detected, indicating that bacteriochlorophyll *a* is not present in strains C15^T and C44.

Genomic DNA was extracted using a commercially available kit (GenElute Plant Genomic DNA miniprep kit; Sigma-Aldrich) according to the manufacturer's instructions.

Nearly full-length 16S rRNA gene sequences (1353/1350 nt) were amplified with the universal eubacterial 16S rRNA gene primers 27F and 1492R (Lane, 1991). PCR amplification was performed with a thermocycler in a total volume of 50 µl. The reaction mixture contained 32 µl

RNase/DNase free water, 5 µl 10 × *Taq* Buffer (containing KCl), 4 µl 25 mM MgCl₂, 5 µl 2 mM dNTPs, 0.9 µl each 10 µM primer, 0.2 µl *Taq* DNA polymerase (5 U µl^{−1}) and 1 µl genomic DNA. PCRs consisted of 33 cycles (45 s at 94 °C, 45 s annealing at 57.3 °C, and 2 min at 72 °C) with an initial denaturation of 3 min at 95 °C and a final elongation step of 15 min at 72 °C.

A partial sequence (471 nt) of the *mxoF* gene was amplified using primers 1003f and 1561r (McDonald *et al.*, 1995). PCR amplification was performed in a total volume of 50 µl; the reaction mixture contained 31.8 µl RNase/DNase free water, 5 µl 10 × *Taq* Buffer (containing KCl), 6 µl 25 mM MgCl₂, 4 µl 2 mM dNTPs, 1 µl each 10 µM primer, 0.2 µl *Taq* DNA polymerase (5 U µl^{−1}) and 1 µl genomic DNA. PCRs consisted of 30 cycles (60 s at 92 °C, 60 s annealing at 55 °C, and 60 s at 72 °C) with an initial denaturation of 3 min at 95 °C and a final elongation step of 5 min at 72 °C.

PCR products were purified with the QIAquick PCR purification system (Qiagen), sequenced with the primers listed above and aligned by using the software packages MEGA4 (Tamura *et al.*, 2007) and CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods using the software package MEGA4 (Tamura *et al.*, 2007). A bootstrap confidence analysis was performed on 1000 replicates to determine the reliability of the tree topology obtained (Felsenstein, 1985). Additionally, multiple alignment of the data and analysis of the sequences were performed using the software package ARB (version December 2007; Ludwig *et al.*, 2004) with the corresponding SILVA SSURF 100 database (release August 2009; Pruesse *et al.*, 2007). Tree reconstruction using the maximum-likelihood method with fastDNAm1 (Olsen *et al.*, 1994) was performed with the ARB software package (see Supplementary Fig. S1, available in IJSEM Online). Comparative 16S rRNA gene sequence analysis indicated that both strains were located within the genus *Methylobacterium*. Sequence similarity calculations and phylogenetic analysis revealed that strains C15^T and C44 were closely related to *M. marchantiae* JT1^T (97.5 %) and *M. jeotgali* S2R03-9^T (97.4 %). A neighbour-joining tree is shown in Fig. 1. Phylogenetic analyses with maximum-likelihood (Supplementary Fig. S1) and maximum-parsimony (results not shown) methods showed slightly different results. The sequence similarity between strains C15^T and C44 was 100 %. A neighbour-joining tree based on the *mxoF* gene is given in Fig. 2.

DNA–DNA hybridization studies were performed according to Ziemke *et al.* (1998) with a hybridization temperature of 73.4 °C after isolation of genomic DNA according to the method of Pitcher *et al.* (1989). DNA–DNA hybridization experiments resulted in DNA–DNA similarity values of 20.4 % (reciprocal 34.6 %) and 11.1 % (reciprocal 28.0 %) in pairing *M. jeotgali* S2R03-9^T with C15^T and C44, respectively, and in DNA–DNA similarity

Fig. 1. Phylogenetic tree reconstruction from a comparative analysis based on 36 individual 16S rRNA gene sequences (1353 nt), available from the European Molecular Biology Laboratory database (accession numbers in parentheses), which shows the relationship between strains C15^T and C44 and members of related taxa. Evolutionary history was inferred using the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) higher than 70 % are shown at nodes. The sequence of *Rhodopseudomonas palustris* DSM 123^T was used as an outgroup. Bar, 1 % sequence dissimilarity.

Fig. 2. Phylogenetic tree reconstruction from a comparative analysis based on 27 partial *mxoF* gene sequences (519 nt), available from the European Molecular Biology Laboratory database (accession numbers in parentheses), which shows the relationship between strains C15^T and C44 and members of related taxa. Evolutionary history was inferred using the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) higher than 70 % are shown at nodes. The sequence of *Hyphomicrobium methylovorum* GM2 was used as an outgroup. Bar, 2 % sequence dissimilarity.

values of 30.5 % (reciprocal 12.3 %) and 5.5 % (reciprocal 12.3 %) in pairing *M. marchantiae* JT1^T with C15^T and C44, respectively. Pairing strain C15^T with strain C44 showed DNA–DNA similarity values of 75.8 % (reciprocal 69.5 %).

Whole-cell fatty acid analysis was carried out using the MIDI protocol as previously described (Kämpfer & Kroppenstedt, 1996), except that cells were grown on M125 at 25 °C prior to fatty acid extraction according to the MIDI protocol. The samples were investigated using a model 5898A microbial identification system (Microbial ID) and the Microbial Identification System standard software (Microbial ID; version TSBA 4.1). The fatty acid profiles of both strains were very similar to that of closely related species of the genus *Methylobacterium* (Table 2), consisting mainly of C_{16:0}, C_{18:1}ω7c and C_{16:1}ω7c/iso-C_{15:0} 2-OH (summed feature 3).

On the basis of the phenotypic differences observed, the results of the DNA–DNA hybridization studies and the differences in 16S rRNA gene sequences, we propose a novel species of the genus *Methylobacterium* with the name *Methylobacterium cerastii* sp. nov. and with strain C15^T as the type strain. As a result of recent work, including DNA–DNA hybridization, we also propose that strain C44 be considered as a strain of *M. cerastii* sp. nov.

Description of *Methylobacterium cerastii* sp. nov.

Methylobacterium cerastii (ce.ras'ti.i. N.L. n. *Cerastium*, a scientific genus name; N.L. gen. n. *cerastii* of *Cerastium*, isolated from *Cerastium holosteoides*).

Aerobic, Gram-stain-negative, rod-shaped and immotile. Cells of strain C15^T are approximately 0.8–1.1 × 1.6–5.4 µm in size and cells of strain C44 are approximately 0.7–1.1 × 2.0–4.9 µm in size. Slow-growing with a growth rate

(µ) of approximately 0.05–0.06 h⁻¹. Grows on R2A, nutrient, PYG, PYE, LB, M65, PCA, K7, NA and TGE media and on M125 supplemented with 0.1–2.0 % methanol (v/v); no growth detected on TS, GP, caso or marine media. Colonies are pinkish coloured, shiny, smooth, circular, with entire margins and a diameter of 1.0–2.0 mm on M125 agar after 7 days of incubation at 25 °C. Pigments have absorption maxima in 80 % (v/v) methanol at 360, 490 and 520 nm, respectively, and a weak peak at 460 nm. Pigments of strain C15^T have an additional absorption maximum in 80 % (v/v) methanol at 314 nm. Produce catalase and oxidase. Strain C15^T grows at 10–28 °C (optimum 20 °C) and pH 4.0–6.0 (optimum 5.0–6.0). Strain C44 grows at 16–28 °C (optimum 20–28 °C) and pH 5.0–7.0 (optimum 6.0). No growth at temperatures below 4 °C or above 36 °C as well as pH greater than 7.2. Growth in medium supplemented with 0.5 % NaCl or higher salt concentrations is not observed. Do not hydrolyse starch but do hydrolyse L-alanine pNP, pNP phenylphosphate, bis-pNP phosphate and pNP phosphate; strain C44 additionally hydrolyses pNP β-D-glucopyranoside. Indole test is negative and Voges–Proskauer test is positive. Urease is produced, but arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not. H₂S is not produced.

Results of tests for carbon-source utilization are summarized in Table 1. Strain C44 can utilize *cis*-aconitate, *trans*-aconitate, adipate, azelate, fumarate, L-glutamate, glutarate, L-histidine, 3-hydroxybenzoate, DL-3-hydroxybutyrate, DL-lactate, L-malate, mesaconate, oxoglutarate, DL-3-phenylacetate, L-phenylalanine, pyruvate, D-ribose, D-sorbitol and suberate as sole carbon source; results are not consistent for citrate and L-arabinose utilization. Strain C15^T can utilize N-acetyl-D-glucosamine, L-glutamate, oxoglutarate and putrescine as sole carbon source; results for L-arabinose

Table 2. Major fatty acid compositions (%) of type strains of species of the genus *Methylobacterium* that are closely related to the novel strains investigated

Taxa: 1, strain C15^T; 2, strain C44; 3, *M. organophilum* LMG 6083^T; 4, *M. jeotgali* S2R03-9^T; 5, *M. phyllosphaerae* DSM 19779^T; 6, *M. oryzae* DSM 18207^T; 7, *M. fujisawaense* DSM 5686^T; 8, *M. marchantiae* JT1^T. All data were obtained comparatively in this study.

Fatty acid	1	2	3	4	5	6	7	8
anteiso-C _{15:0}	1.7							
C _{16:0}	3.9	3.5	3.2	3.4	5.0	5.0	4.8	6.2
C _{18:0}	0.9	2.7	4.6	3.4	3.2	5.4	2.7	1.5
C _{18:1} ω7c*	81.3	76.6	89.5	89.6	87.2	85.1	86.5	74.8
Summed feature 3†	8.4	17.3			2.4		2.7	15.1
Summed feature 2†	1.8		2.8	3.7				2.5
Unknown 14.263‡					2.2	4.6	3.3	
Unknown 14.959‡	2.1							

*For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain.

†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 2 contained one or more of the fatty acids iso-C_{16:1} I and C_{14:0} 3-OH. Summed feature 3 contained one or more of the fatty acids C_{16:1}ω7c and iso-C_{15:0} 2-OH.

‡The unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

are variable. Strain C15^T grows on formamide, but not on formaldehyde, formate or methylamine. Strain C44 does not grow on C₁ compounds other than methanol. Does not grow on methane. Major fatty acids are C_{16:0}, C_{18:1ω7c} and summed feature 3 (C_{16:1ω7c}/iso-C_{15:0} 2-OH). Additionally, strain C15^T contains small amounts of C_{15:0}.

The type strain is C15^T (=DSM 23679^T =CCUG 60040^T =CCM 7788^T), isolated from the leaf surface of *Cerastium holosteoides*.

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