Methylobacterium gnaphalii sp. nov., isolated from leaves of Gnaphalium spicatum

Akio Tani,[1] Nurettin Sahin[2] and Kazuhide Kimbara[3]

Correspondence
Akio Tani
atani@rib.okayama-u.ac.jp

[1]Institute of Plant Science and Resources, Okayama University, Chuo 2-20-1, Kurashiki, 710-0046 Okayama, Japan

[2]Egitim Fakultesi, Mugla University, Kötekli 48000, Mugla, Turkey

[3]Faculty of Engineering, Shizuoka University, Shizuoka 422-8529, Japan

A pink-pigmented, facultatively methylotrophic bacterium, strain 23e[T], was isolated from the leaves of *Gnaphalium spicatum* (cudweed). The cells of strain 23e[T] were Gram-reaction negative, motile and non-spore-forming rods. On the basis of 16S rRNA gene sequence similarities, strain 23e[T] was related to *Methylobacterium organophilum* ATCC 27886[T] (97.1 %) and *Methylobacterium marchantiae* JT1[T] (97 %), and the phylogenetic similarities to all other *Methylobacterium* species with validly published names were less than 97 %. Major cellular fatty acids were $C_{18:1}\omega7c$, $C_{16:00}$ and $C_{18:0}$. The results of DNA–DNA hybridization, phylogenetic analyses based on 16S rRNA and *cpn60* gene sequences, fatty acid profiles, whole-cell matrix-assisted laser desorption/ionization time of flight/MS analysis, physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain 23e[T] from the phylogenetically closest relatives. We propose that strain 23e[T] represents a novel species within the genus *Methylobacterium*, for which the name *Methylobacterium gnaphalii* sp. nov. is proposed. The type strain is 23e[T] (=DSM 24027[T]=NBRC 107716[T]).

The genus *Methylobacterium* consists mostly of pink-pigmented, facultatively methylotrophic members of the class *Alphaproteobacteria*, and at the time of writing, comprises 35 recognized species (http://www.bacterio.cict.fr/m/methylobacterium.html). However, according to Kato *et al.* (2005), *M. chloromethanicum* (McDonald *et al.*, 2001) and *M. dichloromethanicum* (Doronina *et al.*, 2000) are later heterotypic synonyms of *M. extorquens*, and *M. rhodesianum* (Green *et al.*, 1988) is an earlier heterotypic synonym of *M. lusitanum* (Doronina *et al.*, 2002), since they exhibited high levels of DNA–DNA relatedness (69–89 %). Also, the species name 'M. dankookense' (Lee *et al.*, 2009) has been proposed but is currently not validly published. Recently, *M. goesingense* from *Thlaspi goesingense* (Idris *et al.*, 2006), *M. marchantiae* from *Marchantia polymorpha* L. (Schauer *et al.*, 2011), 'M. soli' from soil (Cao *et al.*, 2011), *M. gossipiicola* from the cotton

phyllosphere (Madhaiyan *et al.*, 2012), *M. bullatum* from the surface of a bryophyte gametophyte (Hoppe *et al.*, 2011) and *M. cerastii* from the surface of a leaf (Wellner *et al.*, 2012) were described as new species.

Members of the genus *Methylobacterium* can grow on single-carbon compounds such as methanol, formaldehyde and formate as the sole carbon and energy source, and also on a wide range of multi-carbon growth substances (Green, 1992). Members of the genus *Methylobacterium* are widespread, especially on plant surfaces, where they assimilate methanol emitted from plants as a product of pectin degradation. Recent intensive studies on phyllospheric *Methylobacterium* species showed that members of this genus are the predominant bacterial species on plant surfaces (Delmotte *et al.*, 2009). Recently, we isolated diverse strains of members of the genus *Methylobacterium* from plant leaf samples. We have characterized one of them and proposed the name *M. oxalidis* for the isolate from *Oxalis corniculata* (Tani *et al.*, 2012). Another isolate, strain 23e[T], isolated from *Gnaphalium spicatum*, showed 97.1 % 16S rRNA gene sequence similarity with *M. organophilum*, the closest type strain. Here we describe isolate 23e[T] as a novel species of the genus *Methylobacterium*.

Leaves of *G. spicatum* were collected at the Institute of Plant Science and Resources, Okayama University, in April 2008. Leaves were briefly washed with 50 ml sterile water

and then washed vigorously with 10 ml sterile water. The wash solution was then spread on methanol medium (Tani *et al.*, 2012). After incubation at 28 °C for 3–5 days, a pink-pigmented colony was picked and purified by restreaking on agar plates of the same composition.

Physiological and biochemical tests were carried out at 28 °C. Conventional biochemical tests were performed according to standard methods (Smibert & Krieg, 1994). Oxidation of various substrates was determined by using Biolog GN2 MicroPlates, according to the manufacturer's instructions, and reactions were observed for 2, 3, 5, 7 and 10 days with a microplate reader (Powerscan HT; Dainippon Sumitomo Pharma). The results of the nutritional tests are shown in the species description. Methanol mineral agar medium was also used in tests for the utilization of methylamine (0.1 %, w/v) as the carbon source. Salt tolerance was tested on R2A agar medium supplemented with 2 % (w/v) NaCl. Nitrate reduction was tested in R2A broth containing 0.2 % (w/v) KNO_3.

The 16S rRNA gene of strain $23e^T$ was amplified by PCR, cloned in the pCR-TOPO vector (Invitrogen) and sequenced (Lane, 1991). Sequencing was carried out with an automated DNA sequencer (model 3130; Applied Biosystems). Phylogenetic analysis was performed using MEGA4 software (Tamura *et al.*, 2007), after multiple sequence alignment of the data by CLUSTAL X2 (Larkin *et al.*, 2007). Genetic distances were obtained by the Kimura's two-parameter distance model (Kimura, 1980). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Nei & Kumar, 2000) methods. The robustness for individual branches was estimated by bootstrapping with 1000 replicates (Felsenstein, 1985). Pairwise nucleotide sequence similarity values were calculated by using the algorithm of Myers & Miller (1988), using the EzTaxon server version 2.1 (http://www.eztaxon.org; Chun *et al.*, 2007). The alignment gap was not considered in the similarity calculation.

Pairwise nucleotide similarity calculations after a neighbour-joining analysis indicated that the closest relatives of strain $23e^T$ were *M. organophilum* ATCC 27886^T (97.1 %) and *M. marchantiae* $JT1^T$ (97 %). Strain $23e^T$ showed 16S rRNA gene sequence similarities of below 97.0 % with other members of the genus *Methylobacterium*. The phylogenetic tree based on the 16S rRNA gene sequence, constructed by using the neighbour-joining method, is shown in Fig. 1. The tree inferred by using the maximum-parsimony method also produced similar results (see Fig. S1, available in IJSEM Online).

DNA–DNA hybridization was carried out at 50 °C for 3 h and measured fluorometrically as described by Ezaki *et al.* (1989). The DNA–DNA relatedness between strain $23e^T$ and *M. organophilum* ATCC 27886^T and *M. marchantiae* $JT1^T$ was 26 % in both cases.

The *cpn60* gene was selected for phylogenetic analysis as an alternative marker. The *cpn60* gene sequences of strain $23e^T$ and its closest relatives were determined directly from PCR fragments using the method described by Hill *et al.* (2004). Experimental conditions for PCR amplification and sequencing were the same as described previously (Tani *et al.*, 2012). Strain $23e^T$ showed 92.3 and 92.6 % *cpn60* gene nucleotide sequence similarity with those of *M. organophilum* ATCC 27886^T and *M. marchantiae* $JT1^T$, respectively.

Whole-cell matrix-assisted laser desorption/ionization time of flight (MALDI-TOF)/MS analysis was also performed as described previously (Tani *et al.*, 2012). The results of MALDI-TOF/MS analysis (Fig. S2) showed clearly that strain $23e^T$ has a different spectrum from the phylogenetically closest type strains, *M. organophilum* ATCC 27886^T and *M. marchantiae* $JT1^T$.

The selected physiological and biochemical differential characteristics of strain $23e^T$ are compared with those of related type strains in Table 1. Detailed phenotypic information is given in the species description.

The morphology of cells grown on R2A agar media for 5 days was observed with a confocal laser scanning microscope (FV-1000, Olympus) (Fig. S3). The cell size of strain $23e^T$ was 1.99×0.9 μm, while that of *M. organophilum* ATCC 27886^T was 2.7×1.39 μm and that of *M. marchantiae* $JT1^T$ was 3.1×1.36 μm. The relatively smaller cell size is characteristic of strain $23e^T$.

Fatty acid methyl ester (FAME) analysis of the whole cell was determined by the DSMZ Identification Service using GC (MIDI, Microbial ID). FAMEs were obtained from 40 mg cells, grown aerobically on R2A agar for 3 days at 28 °C and scraped from Petri dishes by saponification, methylation and extraction, using minor modifications of the methods described by Miller (1982) and Kuykendall *et al.* (1988), as noted previously (Tani *et al.*, 2012). The major cellular fatty acids were $C_{18:1}\omega7c$ (83.4 %), $C_{16:00}$ (5.1 %) and $C_{18:00}$ (4.1 %). $C_{18:0}$ 3-OH (2.45 %) was the only hydroxylated fatty acid detected. In addition, a minor amount (0.42 %) of a $C_{12:0}$ fatty acid and an unidentified fatty acid with an equivalent chain length of 11.799 (0.77 %), summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$, 2.55 %) and summed feature 3 (comprising $C_{16:1}\omega7c$ or iso-$C_{15:0}$ 2-OH, 1.22 %), were also detected. Thus, strain $23e^T$ could be distinguished from its phylogenetic relatives based on its fatty acid profile.

Respiratory lipoquinones were extracted from 100 mg freeze-dried cell material based on the two-stage method described by Tindall (1990a, b) and carried out by the Identification Service and Dr Brian Tindall, DSMZ, Braunschweig, Germany. Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (MACHEREY-NAGEL), using hexane : tert-butyl-methylether (9 : 1, v/v) as the solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on an LDC Analytical (Thermo Separation Products) HPLC fitted with a reverse phase column (MACHEREY-NAGEL, 2×125 mm, 3 μm, RP18)
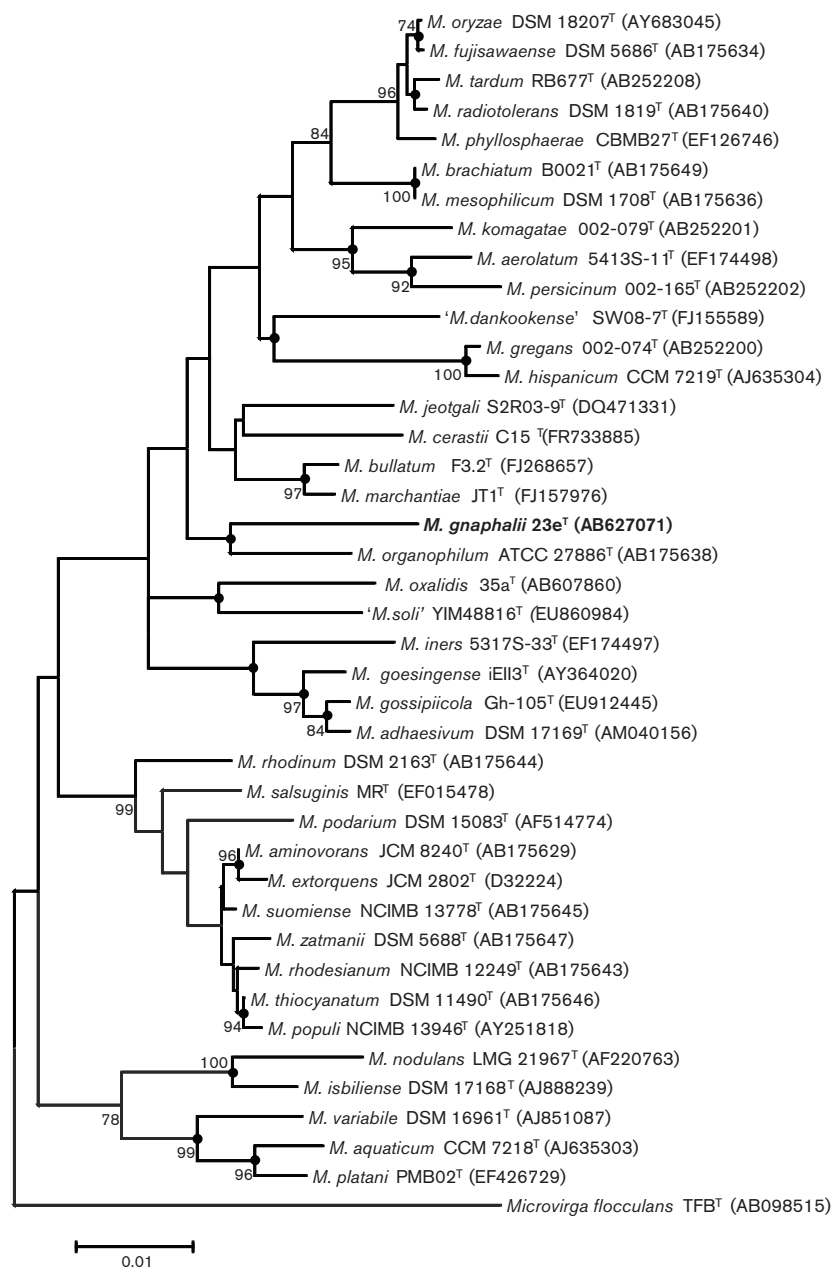
Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences constructed after multiple alignment of data (1291 nt) and clustering with the neighbour-joining method. Bootstrap values greater than 70 % based on 1000 replications are listed as percentages at the branching points. The sequence of *Microvirga flocculans* TFB[T] (AB098515) was used as an out-group. The solid circles indicate corresponding nodes with maximum-parsimony trees. Bar, number of substitutions per nucleotide position.

using methanol as the eluant. Respiratory lipoquinones were detected at 269 nm.

The major ubiquinone system of strains of members of the genus *Methylobacterium* reported to date is ubiquinone Q-10. Similarly, strain 23e[T] had major ubiquinone system Q-10 (95 %) and minor Q-9 (5 %). The occurrence of ubiquinones Q-8, Q-9 and Q-11 as minor components in *M. extorquens*, *M. fujisawaense* and *M. radiotolerans* has been reported by Urakami *et al.* (1993). Ubiquinone system Q-9 has also been reported as a minor (2–7 %) component in type strains of *M. mesophilicum*, *M. komagatae*, *M. brachiatum*, *M. tardum* and *M. hispanicum* (Kato *et al.*, 2008) and 'M. soli' (Cao *et al.*, 2011). Thus strain 23e[T] had

an additional chemotaxonomic difference from its phylogenetic relatives based on the presence of ubiquinone system Q-9.

DNA base composition analysis based on the thermal denaturation temperature, siderophore production and carotenoid extraction and pigment spectral analysis were determined as described by Sahin *et al.* (2008), Schwyn & Neilands (1987) and Sahin (2011), respectively. Members of the genus *Methylobacterium* oxidize methanol to formaldehyde through methanol dehydrogenase (MDH), MDH is a pyrroloquinoline quinone (PQQ)-linked enzyme. It plays an essential role in the first step of methanol oxidation by converting methanol to formaldehyde. In addition, PQQ has

Table 1. Differential characteristics of strain 23e[T] and related species of the genus *Methylobacterium*

Strains: 1, 23e[T]; 2, *M. organophilum* JCM 2833[T] (data from Kato *et al.*, 2005); 3, *M. marchantiae* JT1[T] (Schauer *et al.*, 2011); 4, *M. bullatum* F3.2[T] (Hoppe *et al.*, 2011); 5, *M. jeotgali* S2R03-9[T] (Aslam *et al.*, 2007); 6, *M. cerastii* C15[T] (Wellner *et al.*, 2012); 7, *M. gossipiicola* Gh-105[T] (Madhaiyan *et al.*, 2012); 8, *M. phyllosphaerae* CBMB27[T] (Madhaiyan *et al.*, 2009); 9, *M. platani* PMB02[T] (Kang *et al.*, 2007); 10, *M. oxalidis* 35a[T] (Tani *et al.*, 2012). All strains grew on peptone-rich media and were negative for $C_{16:0}$ 2-OH. +, Positive; −, negative; (+), weakly positive; NA, data not available; V, variable reaction.

Characteristic	1	2	3	4	5	6	7	8	9	10
Isolation source	Leaves of *G. spicatum* L.	Lake sediment	Thallus of a liverwort	Surface of a bryophyte gametophyte	Fermented seafood	Leaf surface	Cotton phyllosphere	Leaf surface of rice	Leaf from a tree	Phyllosphere of *Oxalis corniculata*
Colony pigmentation	Pink	Pink	Red	Red	Non-pigmented	Pinkish	Light pink	Pink	Pink	Pink
Growth on/ at:										
Growth at 35 °C	+	+*	−	−	+*	−	−	−	−	+
Nitrate reduction	(+)	−	(+)	−	+*	NA	−	−	NA	−
2 % NaCl	−	−	−	−	(+)	−	−	−	−	−
Utilization of:										
D-Glucose	−	(+)	−	−	−	−	+	(+)	−	−
Methylamine	−	+*	−	NA	+*	−	−	(+)	NA	−
D-Fructose	+	+	+	+	−	−	(+)	+	+	+
L-Arabinose	−	−	−	NA	−	V	+	+	+	−
D-Xylose	−	+	−	−	+	−	NA	+	NA	−
Citrate	−	−	(+)	V	−	−	+	+	−	−
Hydroxy fatty acids (% of total)										
iso $C_{17:0}$ 3-OH	−	−	−	−	−	−	−	11.5	−	−
$C_{18:0}$ 3-OH	2.5	2.5	−	0.9	−	−	−	0.9	3.46	1.8
Quinone type	Q-10, Q-9	Q-10	NA	NA	Q-10	NA	Q-10	NA	Q-10	Q-10
DNA G+C content (mol%)	67.2	69.6	68	67.1	64.9	NA	64.2	66.8	68.5	70.2

*Data from this study.

a favourable effect on plant growth (Duine & Frank, 1990; Choi *et al.*, 2008). Urakami *et al.* (1992) reported the amount of extracellular PQQ content in strains of members of the genus *Methylobacterium* as between 0.34 and 0.75 µg ml^{-1}, by using methanol as the carbon and energy sources. PQQ production (Tani *et al.*, 2012) and auxin (indole acetate) production were assayed as described by Glickmann & Dessaux (1995). The results are given in the species description.

On the basis of results described above, strain 23e^T represents a novel species within the genus *Methylobacterium*, for which the name *Methylobacterium gnaphalii* sp. nov. is proposed.

Description of *Methylobacterium gnaphalii* sp. nov.

Methylobacterium gnaphalii (gna.pha′li.i. N.L. gen. n. *gnaphalii* of a cudweed *G. spicatum*, referring to the leaves from which the type strain was isolated).

Cells are Gram-reaction negative, motile rods (1.99 × 0.9 µm) and strictly aerobic. Colonies are pink, convex and translucent with regular edges, slow-growing and 0.4 mm in diameter after 5 days on R2A plates at 28 °C. Growth occurs at 28–37 °C. Nitrate reduction is weakly positive. Oxidase negative, catalase positive and other characteristics are given in Table 1. The following substrates produce positive results on Biolog GN2 plates: L-arabinose, methyl pyruvate, acetic acid, formic acid, *β*-hydroxybutyric acid, DL-lactic acid, malonic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid and L-glutamic acid. Methylamine and dimethylamine are not utilized as sole carbon sources. DNase test is negative and urease is positive. Absorbance spectra of the pigment extracts in an acetone–methanol mixture (3 : 1, v/v) have absorbance maxima at 496 and 526 nm. The type strain also has the ability to produce PQQ (24.6 µg ml^{-1}) and indole acetic acid (2.6 µg ml^{-1}). Siderophore production is negative. Ubiquinone Q-10 (95 %) is the predominant isoprenoid quinone, the other is Q-9 (5 %).

The type strain is 23e^T (=DSM 24027^T=NBRC 107716^T). The G + C content of DNA is 67.2 mol% (T_m method).

Acknowledgements

References

Aslam, Z., Lee, C. S., Kim, K. H., Im, W. T., Ten, L. N. & Lee, S. T. (2007). *Methylobacterium jeotgali* sp. nov., a non-pigmented, facultatively methylotrophic bacterium isolated from jeotgal, a traditional Korean fermented seafood. *Int J Syst Evol Microbiol* **57**, 566–571.

Cao, Y. R., Wang, Q., Jin, R. X., Tang, S. K., Jiang, Y., He, W. X., Lai, H. X., Xu, L. H. & Jiang, C. L. (2011). *Methylobacterium soli* sp. nov. a methanol-utilizing bacterium isolated from the forest soil. *Antonie van Leeuwenhoek* **99**, 629–634.

Choi, O., Kim, J., Kim, J. G., Jeong, Y., Moon, J. S., Park, C. S. & Hwang, I. (2008). Pyrroloquinoline quinone is a plant growth promotion factor produced by *Pseudomonas fluorescens* B16. *Plant Physiol* **146**, 657–668.

Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Delmotte, N., Knief, C., Chaffron, S., Innerebner, G., Roschitzki, B., Schlapbach, R., von Mering, C. & Vorholt, J. A. (2009). Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proc Natl Acad Sci U S A* **106**, 16428–16433.

Doronina, N. V., Trotsenko, Y. A., Tourova, T. P., Kuznetsov, B. B. & Leisinger, T. (2000). *Methylopila helvetica* sp. nov. and *Methylobacterium dichloromethanicum* sp. nov. – novel aerobic facultative methylotrophic bacteria utilizing dichloromethane. *Syst Appl Microbiol* **23**, 210–218.

Doronina, N. V., Trotsenko, Y. A., Kuznetsov, B. B., Tourova, T. P. & Salkinoja-Salonen, M. S. (2002). *Methylobacterium suomiense* sp. nov. and *Methylobacterium lusitanum* sp. nov., aerobic, pink-pigmented, facultatively methylotrophic bacteria. *Int J Syst Evol Microbiol* **52**, 773–776.

Duine, J. A. & Frank, J. (1990). The role of PQQ and quinoproteins in methylotrophic bacteria. *FEMS Microbiol Rev* **87**, 221–226.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.

Felsenstein, J. (1985). Confidence limits on phylogenies – an approach using the bootstrap. *Evolution* **39**, 783–791.

Glickmann, E. & Dessaux, Y. (1995). A critical examination of the specificity of the salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl Environ Microbiol* **61**, 793–796.

Green, P. N. (1992). The genus *Methylobacterium*. In *The Prokaryotes*, 2nd edn, pp. 2342–2349. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

Green, P. N., Bousfield, I. J. & Hood, D. (1988). Three new *Methylobacterium* species: *M. rhodesianum* sp. nov., *M. zatmanii* sp. nov., and *M. fujisawaense* sp. nov. *Int J Syst Bacteriol* **38**, 124–127.

Hill, J. E., Penny, S. L., Crowell, K. G., Goh, S. H. & Hemmingsen, S. M. (2004). cpnDB: a chaperonin sequence database. *Genome Res* **14**, 1669–1675.

Hoppe, T., Peters, K. & Schmidt, F. (2011). *Methylobacterium bullatum* sp. nov., a methylotrophic bacterium isolated from *Funaria hygrometrica*. *Syst Appl Microbiol* **34**, 482–486.

Idris, R., Kuffner, M., Bodrossy, L., Puschenreiter, M., Monchy, S., Wenzel, W. W. & Sessitsch, A. (2006). Characterization of Ni-tolerant methylobacteria associated with the hyperaccumulating plant *Thlaspi goesingense* and description of *Methylobacterium goesingense* sp. nov. *Syst Appl Microbiol* **29**, 634–644.

Kang, Y.-S., Kim, J., Shin, H.-D., Nam, Y.-D., Bae, J.-W., Jeon, C. O. & Park, W. (2007). *Methylobacterium platani* sp. nov., isolated from a leaf of the tree *Platanus orientalis*. *Int J Syst Evol Microbiol* **57**, 2849–2853.

Kato, Y., Asahara, M., Arai, D., Goto, K. & Yokota, A. (2005). Reclassification of *Methylobacterium chloromethanicum* and *Methylobacterium dichloromethanicum* as later subjective synonyms of

Methylobacterium extorquens and of *Methylobacterium lusitanum* as a later subjective synonym of *Methylobacterium rhodesianum*. *J Gen Appl Microbiol* **51**, 287–299.

Kato, Y., Asahara, M., Goto, K., Kasai, H. & Yokota, A. (2008). *Methylobacterium persicinum* sp. nov., *Methylobacterium komagatae* sp. nov., *Methylobacterium brachiatum* sp. nov., *Methylobacterium tardum* sp. nov. and *Methylobacterium gregans* sp. nov., isolated from freshwater. *Int J Syst Evol Microbiol* **58**, 1134–1141.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradorhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lane, D. J. (1991). 16S/23S sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A. & other authors (2007). CLUSTAL W and CLUSTAL_X version 2.0. *Bioinformatics* **23**, 2947–2948.

Lee, S. W., Oh, H. W., Lee, K. H. & Ahn, T. Y. (2009). *Methylobacterium dankookense* sp. nov., isolated from drinking water. *J Microbiol* **47**, 716–720.

Madhaiyan, M., Poonguzhali, S., Kwon, S.-W. & Sa, T.-M. (2009). *Methylobacterium phyllosphaerae* sp. nov., a pink-pigmented, facultative methylotroph from the phyllosphere of rice. *Int J Syst Evol Microbiol* **59**, 22–27.

Madhaiyan, M., Poonguzhali, S., Senthilkumar, M., Lee, J. S. & Lee, K. C. (2012). *Methylobacterium gossipiicola* sp. nov., a pink-pigmented, facultatively methylotrophic bacterium isolated from the cotton phyllosphere. *Int J Syst Evol Microbiol* **62**, 162–167.

McDonald, I. R., Doronina, N. V., Trotsenko, Y. A., McAnulla, C. & Murrell, J. C. (2001). *Hyphomicrobium chloromethanicum* sp. nov. and *Methylobacterium chloromethanicum* sp. nov., chloromethane-utilizing bacteria isolated from a polluted environment. *Int J Syst Evol Microbiol* **51**, 119–122.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Myers, E. W. & Miller, W. (1988). Optimal alignments in linear space. *Comput Appl Biosci* **4**, 11–17.

Nei, M. & Kumar, S. (2000). *Molecular Evolution and Phylogenetics*. New York: Oxford University Press.

Sahin, N. (2011). Significance of absorption spectra for the chemotaxonomic characterization of pigmented bacteria. *Turk J Biol* **35**, 167–175.

Sahin, N., Kato, Y. & Yilmaz, F. (2008). Taxonomy of oxalotrophic *Methylobacterium* strains. *Naturwissenschaften* **95**, 931–938.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Schauer, S., Kämpfer, P., Wellner, S., Spröer, C. & Kutschera, U. (2011). *Methylobacterium marchantiae* sp. nov., a pink-pigmented, facultatively methylotrophic bacterium isolated from the thallus of a liverwort. *Int J Syst Evol Microbiol* **61**, 870–876.

Schwyn, B. & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* **160**, 47–56.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt. Washington, DC: American Society for Microbiology.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tani, A., Sahin, N. & Kimbara, K. (2012). *Methylobacterium oxalidis* sp. nov., isolated from leaves of *Oxalis corniculata*. *Int J Syst Evol Microbiol* **62**, 1647–1652.

Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Urakami, T., Yashima, K., Kobayashi, H., Yoshida, A. & Ito-Yoshida, C. (1992). Production of pyrroloquinoline quinone by using methanol-utilizing bacteria. *Appl Environ Microbiol* **58**, 3970–3976.

Urakami, T., Araki, H., Suzuki, K.-I. & Komagata, K. (1993). Further studies of the genus *Methylobacterium* and description of *Methylobacterium aminovorans* sp. nov. *Int J Syst Bacteriol* **43**, 504–513.

Wellner, S. A., Lodders, N. & Kämpfer, P. (2012). *Methylobacterium cerastii* sp. nov., isolated from the leaf surface of *Cerastium holosteoides*. *Int J Syst Evol Microbiol* **62**, 917–924.