# *Methylobacterium haplocladii* sp. nov. and *Methylobacterium brachythecii* sp. nov., isolated from bryophytes

Akio Tani[1] and Nurettin Sahin[2]

[1]Institute of Plant Science and Resources, Okayama University, Okayama, Japan

[2]Egitim Fakultesi, Mugla Sitki Kocman University, 48170 Kotekli, Mugla, Turkey

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

Pink-pigmented, facultatively methylotrophic bacteria, strains 87e[T] and 99b[T], were isolated from the bryophytes *Haplocladium microphyllum* and *Brachythecium plumosum*, respectively. The cells of both strains were Gram-reaction-negative, motile, non-spore-forming rods. On the basis of 16S rRNA gene sequence similarity, strains 87e[T] and 99b[T] were found to be related to *Methylobacterium organophilum* ATCC 27886[T] (97.1 % and 97.7 %, respectively). Strains 87e[T] and 99b[T] showed highest 16S rRNA gene similarity to *Methylobacterium gnaphalii* 23e[T] (98.3 and 99.0 %, respectively). The phylogenetic similarities to all other species of the genus *Methylobacterium* with validly published names were less than 97 %. Major cellular fatty acids of both strains were $C_{18:1}\omega7c$ and $C_{18:0}$. The results of DNA–DNA hybridization, phylogenetic analyses based on 16S rRNA and *cpn*60 gene sequences, fatty acid profiles, whole-cell matrix-assisted, laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analysis, and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strains 87e[T] and 99b[T] from their phylogenetically closest relatives. We propose that strains 87e[T] and 99b[T] represent novel species within the genus *Methylobacterium*, for which the names *Methylobacterium haplocladii* sp. nov. (type strain 87e[T]=DSM 24195[T]=NBRC 107714[T]) and *Methylobacterium brachythecii* sp. nov. (type strain 99b[T]=DSM 24105[T]=NBRC 107710[T]) are proposed.

The genus *Methylobacterium* consists mostly of pink-pigmented, facultative methylotrophs belonging to class *Alphaproteobacteria*, and at the time of writing, comprises 37 recognized species (http://www.bacterio.net/m/methylobacterium.html). Recently described species include *Methylobacterium longum* (Knief *et al.*, 2012), *Methylobacterium bullatum* (Hoppe *et al.*, 2011), *Methylobacterium gossipiicola* (Madhaiyan *et al.*, 2012), *Methylobacterium cerastii* (Wellner *et al.*, 2012), *Methylobacterium marchantiae* (Schauer *et al.*, 2011) and *Methylobacterium oxalidis* (Tani *et al.*, 2012a), while *Methylobacterium soli* (Cao *et al.*, 2011), '*Methylobacterium funariae*' (Schauer & Kutschera, 2011) and *Methylobacterium gnaphalii* (Tani *et al.*, 2012b) have been published online.

Species of the genus *Methylobacterium* can grow on single-carbon compounds such as methanol, formaldehyde and formate as 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 (Fall & Benson, 1996). Recently we isolated members of the genus *Methylobacterium* from plant leaf samples to evaluate their diversity. Their whole-cell mass spectrometry data (WC-MS) obtained by matrix-assisted, laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) were used to discriminate different species (Tani *et al.*, 2012c). The WC-MS-based classification allowed rapid identification and classification without sequencing any marker genes, and consequently unique strains could be easily identified within hundreds of isolates. We have characterized two such unique isolates and proposed *Methylobacterium oxalidis* isolated from *Oxalis corniculata* (Tani *et al.*, 2012a) and *Methylobacterium gnaphalii* isolated from *Gnaphalium spicatum* (Tani *et al.*, 2012b). Here we describe a further two unique isolates, 87e[T]

and 99b[T], as representative of the novel species *Methylobacterium haplocladii* sp. nov. and *Methylobacterium brachythecii* sp. nov., respectively.

Bryophyte samples, *Haplocladium microphyllum* and *Brachythecium plumosum* were collected at the Institute of Plant Science and Resources, Okayama University, in April 2008. Isolation procedures and media used were reported previously (Tani *et al.*, 2012a). Physiological and biochemical tests were carried out at 28 °C. Conventional biochemical tests were done according to standard methods (Smibert & Krieg, 1994). Oxidation of various substrates was determined by using Biolog GN2 MicroPlates, as described previously (Tani *et al.*, 2012a). Utilization of methylamine (0.1 %, w/v), salt tolerance and nitrate reduction with 0.2 % KNO$_3$ (w/v) were examined as described previously (Tani *et al.*, 2012a). The results of the nutritional tests are given in the species description.

The 16S rRNA gene of strains 87e[T] and 99b[T] was PCR-amplified, cloned in pCR-TOPO vector (Invitrogen), sequenced (Lane, 1991) and analysed using MEGA5 software (Tamura *et al.*, 2011) after multiple alignment of data by the CLUSTALx2 program (Larkin *et al.*, 2007). Genetic distances were obtained by the Kimura's two-parameter distance model (Kimura, 1980). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Nei & Kumar, 2000) and maximum-likelihood methods in PhyML (Guindon & Gascuel, 2003). 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 the neighbour-joining analysis indicated that the closest relatives of strain 87e[T] included *Methylobacterium organophilum* ATCC 27886[T] (97.1 %) and *Methylobacterium marchantiae* JT1[T] (96.9 %) and those of strain 99b[T] were *Methylobacterium organophilum* ATCC 27886[T] (97.7 %) and *Methylobacterium marchantiae* JT1[T] (96.9 %). Strains 87e[T] and 99b[T] showed highest 16S rRNA gene similarity to *Methylobacterium gnaphalii* 23e[T] (98.3 % and 99.0 %, respectively). The two strains showed 16S rRNA gene sequence similarity below 97.0 % with other members of the genus *Methylobacterium*. The phylogenetic tree based on 16S rRNA gene sequence reconstructed by using the neighbour-joining method is shown in Fig. 1. Parsimony and maximum-likelihood trees also produced similar results (see Figs S1 and S2, available in IJSEM Online). Thus, the two strains are close relatives of *Methylobacterium gnaphalii* 23e[T], and their sequences showed 98.3 % identity to each other.

DNA–DNA hybridization between closely related strains was carried out at 50 °C for 3 h and measured fluorometrically as described by Ezaki *et al.* (1989). The DNA–DNA relatedness values obtained were 29.3 % (87e[T] and

*Methylobacterium organophilum* NBRC 15689[T]), 22.4 % (99b[T] and *Methylobacterium organophilum* NBRC 15689[T]), 34.6 % (87e[T] and *Methylobacterium gnaphalii* 23e[T]), 30.8 % (99b[T] and *Methylobacterium gnaphalii* 23e[T]) and 37.3 % (99b[T] and 87e[T]) (Table S1).

The *cpn*60 gene was selected for phylogenetic analysis as an alternative marker. Experimental conditions are the same as previously described (Tani *et al.*, 2012a). Strain 87e[T] showed 91.9 % (*Methylobacterium organophilum* NBRC 15689[T]), 95.0 % (*Methylobacterium marchantiae* JT1[T]), 95.7 % (strain 99b[T]) and 94.6 % (*Methylobacterium gnaphalii* 23e[T]) *cpn60* gene nucleotide sequence similarity. Strain 99b[T] showed 92.1 % (*Methylobacterium organophilum* NBRC 15689[T]), 93.9 % (*Methylobacterium marchantiae* JT1[T]) and 96.8 % (*Methylobacterium gnaphalii* 23e[T]) *cpn60* gene nucleotide sequence similarity.

The results of whole-cell MALDI-TOF/MS analysis showed that strains 87e[T] and 99b[T] have clearly different spectra from their closest relatives (Fig. S3). *Methylobacterium marchantiae* JT1[T], *Methylobacterium gnaphalii* 23e[T] and strain 99b[T] share an identical most prominent peak at *m/z* 8355.2, but most of the other peaks were not found in common.

The selected physiological and biochemical differential characteristics of strains 87e[T] and 99b[T] are compared with those of related type strains in Table 1. Detailed phenotypic descriptions are given in the species descriptions.

Fatty acid methyl ester (FAME) analysis of whole cells was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany) identification service using GC (MIDI; Microbial ID). FAMEs were obtained from 40 mg cells grown aerobically on R2A agar at 28 °C after 9 days for strain 87e[T] and 3 days for strain 99b[T] as described previously (Tani *et al.*, 2012a). Major cellular fatty acids of strain 87e[T] were $C_{18:1}\omega 7c$ (83.1 %), $C_{18:0}$ (7.15 %) and $C_{16:0}$ (3.0 %). Summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$; 1.6 %) and summed feature 3 (comprising $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH; 5.2 %) were also detected. Major cellular fatty acids of strain 99b[T] were $C_{18:1}\omega 7c$ (85.5 %), $C_{18:0}$ (5.92 %) and $C_{16:0}$ (2.7 %). Summed feature 2 (comprising $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$; 1.9 %) and summed feature 3 (comprising $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH; 2.2 %) were also detected. $C_{18:0}$ 3-OH (1.85 %) was the only hydroxylated fatty acid detected. Thus, strain 87e[T] can be distinguished from its closest phylogenetic relatives based on its lack of $C_{18:0}$ 3-OH fatty acid (Table 1).

Respiratory lipoquinones were extracted from 100 mg of freeze-dried cell material based on the two-stage method described by Tindall (1990a, b) and analysis was carried out by the Identification Service of the DSMZ as described previously (Tani *et al.*, 2012a). The major ubiquinone system of strains belonging to the genus *Methylobacterium* reported to date is ubiquinone Q-10. The quinone group of
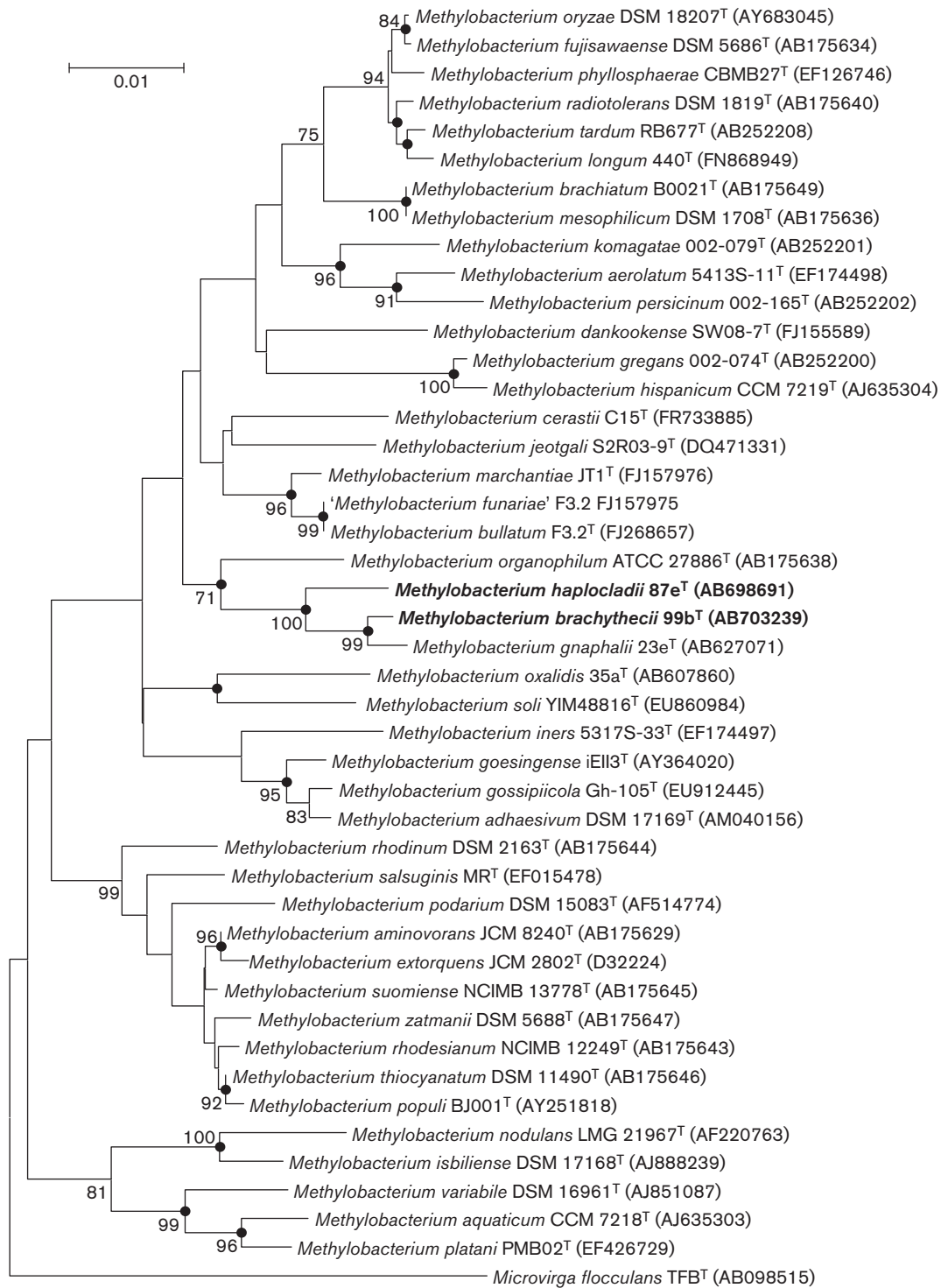
**Fig. 1.** Phylogenetic analysis based on 16S rRNA gene sequences reconstructed after multiple alignment of data (1291 nt) and clustering with neighbour-joining method. Bootstrap values greater than 70 % based on 1000 replications are listed as percentages at the branching points. Bar, number of substitutions per nucleotide position. The sequence of *Microvirga flocculans* TFB[T] (AB098515) was used as an outgroup. Solid circles indicate that corresponding nodes were seen in both the maximum-parsimony and maximum-likelihood alternative treeing methods.

**Table 1.** Differential characteristics of strains 87e[T], 99b[T] and related type strains of species of the genus *Methylobacterium*

Strains: 1, *Methylobacterium haplocladii* sp. nov. 87e[T]; 2, *Methylobacterium brachythecii* sp. nov. 99b[T]; 3, *Methylobacterium gnaphalii* 23e[T] (data from Tani *et al.*, 2012b); 4, *Methylobacterium organophilum* JCM 2833[T] (Kato *et al.* 2005); 5, *Methylobacterium marchantiae* JT1[T] (Schauer *et al.*, 2011); 6, *Methylobacterium bullatum* F3.2[T] (Hoppe *et al.*, 2011); 7, *Methylobacterium jeotgali* S2R03-9[T] (Aslam *et al.* 2007); 8, *Methylobacterium cerastii* C15[T] (Wellner *et al.*, 2012); 9, *Methylobacterium gossipiicola* Gh-105[T] (Madhaiyan *et al.*, 2012); 10, *Methylobacterium phyllosphaerae* CBMB27[T] (Madhaiyan *et al.*, 2009); 11, *Methylobacterium platani* PMB02[T] (Kang *et al.*, 2007); and 12, *Methylobacterium oxalidis* 35a[T] (Tani *et al.*, 2012a). +, Positive; −, negative; (+), weakly positive; NA, data not available; V, variable reaction; *, data from this study. *Hm*, *Haplocladium microphyllum*; *Bp*, *Brachythecium plumosum*; *Gs*, leaves of *Gnaphalium spicatum* L.; Lake, lake sediment; Thallus, thallus of a liverwort; BG, surface of a bryophyte gametophyte; FS, fermented seafood; Leaf, leaf surface; PC, cotton phyllosphere; LR, leaf surface of rice; LT, leaf from a tree; PO, phyllosphere of *Oxalis corniculata*.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolation source | *Hm* | *Bp* | *Gs* | Lake | Thallus | BG | FS | Leaf | PC | LR | LT | PO |
| Colony pigmentation | Light pink | Light pink | Pink | Pink | Red | Red | Non-pigmented | Pinkish | Light pink | Pink | Pink | Pink |
| Growth on peptone rich media | − | + | + | + | + | + | + | + | + | + | + | + |
| Growth at 35 °C | − | − | + | +* | − | − | +* | − | − | − | − | + |
| Nitrate reduction | − | (+) | (+) | − | (+) | − | +* | NA | − | − | NA | − |
| Growth with 2 % NaCl | − | − | − | − | − | − | (+) | − | − | − | − | − |
| Utilization of: | | | | | | | | | | | | |
| D-Glucose | − | + | − | (+) | − | − | − | − | + | (+) | − | − |
| Methylamine | − | + | − | +* | − | 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 | − | 1.85 | 2.5 | 2.5 | − | 0.9 | − | − | − | 0.9 | 3.46 | 1.8 |
| Quinone type | Q$_{10}$ | Q$_{10}$ | Q$_{10}$, Q$_9$ | Q$_{10}$ | NA | NA | Q$_{10}$ | NA | Q$_{10}$ | NA | Q$_{10}$ | Q$_{10}$ |
| DNA G+C content (mol%) | 67.3 | 66.5 | 67.2 | 69.6 | 68 | 67.1 | 64.9 | NA | 64.2 | 66.8 | 68.5 | 70.2 |

strain 87e[T] was Q-10 (100 %) and that of strain 99b[T] was also Q-10 (100 %).

DNA base composition analysis was done based on thermal denaturation temperature (Sahin *et al.*, 2008). A siderophore assay was performed according to the published method of Schwyn & Neilands (1987). Pyrroloquinoline quinone (PQQ) content (Tani *et al.*, 2012a) and indole acetate content were measured as reported by Glickmann & Dessaux (1995). Carotenoid extraction and pigment spectral analysis were determined according to Sahin (2011). The results are given in the species description.

On the basis of results described above, strains 87e[T] and 99b[T] represent novel species within the genus *Methylobacterium*, for which the names *Methylobacterium haplocladii* sp. nov., and *Methylobacterium brachythecii* sp. nov., respectively, are proposed.

## Description of *Methylobacterium haplocladii* sp. nov.

*Methylobacterium haplocladii* (hap.lo.cla′di.i. N.L. gen. n. haplocladii of the epilithic moss *Haplocladium microphyllum*, referring to the plant from which the type strain was isolated).

Cells are Gram-reaction negative, motile, rods (2.5 × 1.1 μm) and strictly aerobic. Colonies are light pink, convex and translucent with regular edges, very slow-growing and 0.1 mm in diameter after 9 days on R2A plates at 28 °C. Growth occurs at 28 °C and not at 32 °C. Nitrate reduction is negative and motility is negative. Oxidase-negative and catalase-positive; other characteristics are given in Table 1. The following substrates produce positive results in Biolog GN2 plates: α-D-Glucose, L-rhamnose, turanose, methyl pyruvate, formic acid, β-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, bromosuccinic acid and glycerol. Methylamine is not utilized as sole carbon sources. DNase and urease are negative. Absorbance spectra of the pigment extracts in acetone/methanol (3 : 1, v/v) have absorbance maxima at 384, 470, 496 and 528 nm. Type strain has the ability to produce indole acetate (0.7 μg ml$^{-1}$). PQQ production is not observed due to poor growth on methanol. Siderophore production is negative. Ubiquinone Q-10 is the only detected isoprenoid quinone. Major cellular fatty acids are C$_{18:1}$ω7c, C$_{18:0}$ and C$_{16:0}$.

The type strain is 87e$^T$ (=DSM 24195$^T$=NBRC 107714$^T$), isolated from *Haplocladium microphyllum* collected on the premises of the Institute of Plant Science and Resources, Okayama University, Japan. The G+C content of DNA of the type strain is 67.3 mol% (T$_m$ method).

## Description of *Methylobacterium brachythecii* sp. nov.

*Methylobacterium brachythecii* (bra.chy.the′ci.i. N.L. gen. n. *brachythecium* of Rusty feather-moss *Brachythecium plumosum*, referring to the plant from which the type strain was isolated).

Cells are Gram-reaction-negative, motile, rods (2.5× 1.2 µm) and strictly aerobic. Colonies are light pink, convex and translucent with regular edges, and 0.2 mm in diameter after 5 days on R2A plates at 28 °C. Growth occurs at 28 °C and not at 32 °C. Nitrate reduction is weakly positive and motility is positive. Oxidase-positive and catalase-positive; other characteristics are given in Table 1. The following substrates produce positive results in Biolog GN2 plates: dextrin, glycogen, L-arabinose, α-D-glucose, turanose, methyl pyruvate, acetic acid, formic acid, β-hydroxybutyric acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, D-saccharic acid, succinic acid, bromosuccinic acid, succinamic acid, L-asparagine, L-aspartic acid, L-glutamic acid, glycerol and DL-α-glycerol-phosphate. Methylamine is utilized as a sole carbon source. DNase is negative and urease is positive. Absorbance spectra of the pigment extracts in acetone/methanol (3:1, v/v) have absorbance maxima at 472, 496 and 528 nm. Type strain has the ability to produce PQQ (13.4 µg ml$^{-1}$) and indole acetate (1.3 µg ml$^{-1}$). Siderophore production is negative. Ubiquinone Q-10 is the only detected isoprenoid quinone. Major cellular fatty acids are C$_{18:1}\omega 7c$, C$_{18:0}$ and C$_{16:0}$. C$_{18:0}$ 3-OH hydroxylated fatty acid was also detected.

The type strain is 99b$^T$ (=DSM 24105$^T$=NBRC 107710$^T$), isolated from *Brachythecium plumosum* collected on the premises of the Institute of Plant Science and Resources, Okayama University, Japan. The G+C content of DNA of the type strain is 66.5 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.

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.

Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution 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.

Fall, R. & Benson, A. A. (1996). Leaf methanol - the simplest natural product from plants. *Trends Plant Sci* **1**, 296–301.

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.

Guindon, S. & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* **52**, 696–704.

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

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.

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.

Knief, C., Dengler, V., Bodelier, P. L. & Vorholt, J. A. (2012). Characterization of *Methylobacterium* strains isolated from the phyllosphere and description of *Methylobacterium longum* sp. nov. *Antonie van Leeuwenhoek* **101**, 169–183.

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.

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.

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. & Kutschera, U. (2011).** A novel growth-promoting microbe, *Methylobacterium funariae* sp. nov., isolated from the leaf surface of a common moss. *Plant Signal Behav* **6**, 510–515.

**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, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

**Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011).** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

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

**Tani, A., Sahin, N. & Kimbara, K. (2012b).** *Methylobacterium gnaphalii* sp. nov., isolated from leaves of *Gnaphalium spicatum*. *Int J Syst Evol Microbiol* **62**, 2602–2607.

**Tani, A., Sahin, N., Matsuyama, Y., Enomoto, T., Nishimura, N., Yokota, A. & Kimbara, K. (2012c).** High-throughput identification and screening of novel *Methylobacterium* species using whole-cell MALDI-TOF/MS analysis. *PLoS ONE* **7**, e40784.

**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.

**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.