Methylobacterium persicinum sp. nov.,
Methylobacterium komagatae sp. nov.,
Methylobacterium brachiatum sp. nov.,
Methylobacterium tardum sp. nov. and
Methylobacterium gregans sp. nov., isolated from
 freshwater

Yuko Kato,¹ Mika Asahara,¹ Keiichi Goto,¹ Hiroaki Kasai²
 and Akira Yokota³

Correspondence

Yuko Kato

ytanaka@mitsui-norin.co.jp

¹Microbiological and Analytical Group, Food Research Laboratories, Mitsui Norin Co. Ltd, 223-1, Miyahara, Fujieda, Shizuoka 426-0133, Japan

²Marine Biotechnology Institute Co. Ltd, 3-75-1, Heita, Kamaishi, Iwate 026-0001, Japan

³Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1, Yayoi 1-chome, Bunkyo-ku, Tokyo 113-0032, Japan

Eight strains, 002-165^T, 002-079^T, B0021^T, Hojyo2, RB603B, RB677^T, 002-074^T and RB678, isolated from the environment of food-processing factories in Japan, were characterized using a polyphasic approach. The isolates were Gram-negative, strictly aerobic, pink-pigmented, facultatively methylotrophic, non-spore-forming rods. The chemotaxonomic characteristics of these isolates included the presence of C_{18:1}ω7c as the major cellular fatty acid and ubiquinone Q-10 as the predominant ubiquinone. The DNA G+C content was 67.1–71.1 mol%. Phylogenetic analyses of 16S rRNA and DNA gyrase B subunit (*gyrB*) nucleotide sequence confirmed that the eight strains belonged to the *Methylobacterium* clade. Moreover, a DNA–DNA hybridization analysis showed that the eight isolates represented five novel species. On the basis of their phenotypic and phylogenetic distinctiveness, the isolates represent five novel species within the genus *Methylobacterium*, for which the names *Methylobacterium persicinum* sp. nov. (type strain 002-165^T = DSM 19562^T = NBRC 103628^T = NCIMB 14378^T), *Methylobacterium komagatae* sp. nov. (type strain 002-079^T = DSM 19563^T = NBRC 103627^T = NCIMB 14377^T), *Methylobacterium brachiatum* sp. nov. (type strain B0021^T = DSM 19569^T = NBRC 103629^T = NCIMB 14379^T), *Methylobacterium tardum* sp. nov. (type strain RB677^T = DSM 19566^T = NBRC 103632^T = NCIMB 14380^T) and *Methylobacterium gregans* sp. nov. (type strain 002-074^T = DSM 19564^T = NBRC 103626^T = NCIMB 14376^T) are proposed.

The genus *Methylobacterium* was first described by Patt *et al.* (1976) and, at the time of writing, comprises 22 recognized species. Members of the genus are aerobic, Gram-negative, pink-pigmented, facultatively methylotrophic bacteria. They have been recovered from lake

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA (*rrs*) and gyrase B subunit (*gyrB*) gene sequences of the novel strains are AB175649, AB252200–AB252202, AB252205 and AB252207–AB252209 (*rrs*) and AB302916–AB302918, AB302920, AB302922–AB302924 and AB302926 (*gyrB*), as detailed in Figs 1 and 2.

A similarity matrix for 16S rRNA and *gyrB* gene sequences of the isolates and their neighbours is available as supplementary material with the online version of this paper.

sediment, soil, sewage, plants, rice grains and a fermenter. Recently, five novel species affiliated to the genus, *Methylobacterium hispanicum*, *Mtb. aquaticum*, *Mtb. variabile*, *Mtb. isbiliense* and *Mtb. adhaesivum*, were isolated from drinking-water systems (Gallego *et al.*, 2005a, b, c, 2006). In the environment and in food-manufacturing plants, pink-pigmented oligotrophic bacteria are often isolated, and manufacturers have had trouble with these bacteria in terms of product contamination. During the investigation of microbial contamination in food-processing environments, eight strains (002-165^T, 002-079^T, B0021^T, Hojyo2, RB603B, RB677^T, 002-074^T and RB678) were found to represent potentially distinct *Methylobacterium* species on the basis of analyses of partial 5'

sequences of the 16S rRNA gene (Goto *et al.*, 2000). The aim of the present study was to resolve the taxonomic positions of these eight isolates as well as unidentified strain DSM 1857 (Gräf & Bauer, 1973), isolated from a tap-water system, using a combination of taxonomic approaches.

Strains 002-165^T, 002-079^T, B0021^T, Hojyo2, RB603B, RB677^T, 002-074^T and RB678 were isolated from water samples taken from food-manufacturing plants by dilution plating on R2A media (Merck) at 30 °C for 3 days. Pink-pigmented colonies were isolated on the plate and subsequently purified four times on R2A medium at 30 °C. Type/reference strains of *Methylobacterium* species were obtained from the DSMZ, IAM, JCM and NCIMB. The strains were stored at -80 °C in trypticase soy broth (Nissui) supplemented with 15% (v/v) glycerol until further analysis. All strains were grown on nutrient agar plates at 30 °C for 3 days.

Morphological characteristics were observed by light microscopy (BH2 microscope; Olympus) after 6 days growth on glycerol-peptone (GP) medium containing 1.0% (v/v) glycerol, 1.0% (w/v) peptone (Difco) and 1.5% (w/v) agar noble (Difco) at 30 °C. Utilization of sole carbon sources in mineral salts liquid medium at 30 °C was investigated by using the method of Green *et al.* (1992). The results were read after 14 days incubation, in comparison with positive (methanol and sodium pyruvate) and negative controls. The effects of temperature on growth were examined using mineral salts liquid medium, with 0.1% (v/v) methanol or 0.1% (w/v) sodium pyruvate as the sole carbon and energy source.

The 16S rRNA gene was analysed as described by Kato *et al.* (2005). *gyrB* gene sequences were determined directly from PCR fragments using the method described by Yamamoto & Harayama (1995) and Kasai *et al.* (2000). The primers used for *gyrB* amplifications were UP-1M (forward: 5'-GAGGTCATCATGACCCAGCTSCAYGCCGCGGWAA-GTTCGAC-3') and UP-2r (reverse: 5'-AGCAGGGTACGGATGTGCGAGCCRTCNACRTCNCGTCAT-3'). Primer UP-1M annealed to positions 274–315 and primer UP-2r annealed at positions 1486–1529 (relative to *Escherichia coli* K-12 gene numbering). The amplified products were purified by gel electrophoresis on 0.8% low-melting-point agarose (SeaPlaque GTG; FMC Bioproducts). The purified fragments were recovered from the agarose by using a QIAquick gel extraction kit (Qiagen). Sequencing was carried out with an ABI PRISM Dye Terminator cycle sequencing kit with sequencing primers UP1Ms (forward: 5'-GAGGTCATCATGACCCAGCTSCA-3'; positions 274–296), INVF2 m (forward: 5'-TGGTGAACGAYTCSTTCAACGAGA-3'; positions 769–793), INVR (reverse: 5'-ACSAGYTTGTCC-TTSGTCTGSGAG-3'; positions 1018–1044) and UP2rs (reverse: 5'-AGCAGGGTACGGATGTGCGAGCC-3'; positions 1507–1529). The products were analysed by an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems) according to the manufacturer's instructions. Phylogenetic trees

were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods using MEGA version 3.1 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985). Alignment analysis was carried out using CLUSTAL W (Thompson *et al.*, 1994), and gaps and unidentified base positions were edited using BioEdit (Hall, 1999). *gyrB* gene sequences and their deduced amino acid sequences were aligned and corrected manually. Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1980).

Fatty acid profiles, G+C contents and DNA–DNA relatedness values were analysed using methods described previously (Kato *et al.*, 2005). Quinones were extracted from freeze-dried cells according to Sano *et al.* (1996) and analysed by using an Alliance HPLC system equipped with a Waters 996 Photodiode Array detector. A Mightysil RP-18 column (4.6 × 250 mm; Kanto Chemical) was used for separation and methanol/propan-2-ol (3:1, v/v) was used as the mobile phase. Quinones were identified by their UV spectra.

The eight isolates were Gram-negative, forming straight to ovoid (1.0–2.1 × 1.9–5.2 µm), slightly curved rods or branched cells, and were motile. Cells occurred singly or occasionally in pairs or rosettes. They were strictly aerobic and oxidase and catalase reactions were positive. Colonies were 0.4–2.2 mm in diameter, light pink, vermilion or red, circular and convex with entire, smooth edges after 9 days incubation on GP agar. Exceptionally, the colony surfaces of strains Hojyo2 and RB603B were rough, wrinkled and tough. Cells of the strains often contained poly-β-hydroxybutyrate and volutin granules.

Differential phenotypic and chemotaxonomic characteristics between the eight strains and related *Methylobacterium* species are summarized in Table 1. All eight strains could utilize methanol, citrate, pyruvate and L-glutamate as sole carbon and energy sources, but could not utilize N,N-dimethylformamide, monomethylamine or trimethylamine. The methanol utilization abilities of strains 002-165^T and 002-079^T were weak.

The predominant cell fatty acid was C_{18:1ω7c} (75.8–88.5%), with smaller amounts of C_{18:0} (3.2–14.1%), C_{16:1ω7c} (1.6–6.1%), C_{16:0} iso (<0.5–4.8%) and C_{15:0} anteiso (0.7–1.5%). Unusually, strain RB677 contained the hydroxylated fatty acid C_{10:0} 3-OH (2.0%). The G+C contents of the isolates were 67.1–71.1 mol%. The major respiratory quinone was ubiquinone Q-10 (93.5–100%), and some strains contained low levels (<6.5%) of Q-9.

Nearly complete 16S rRNA gene sequences (1433–1436 nt) and partial *gyrB* sequences (1176–1196 nt) were determined (see Supplementary Table S1 in IJSEM Online). A phylogenetic tree based on the 16S rRNA gene (Fig. 1) indicated that our isolates and DSM 1857 formed a coherent and monophyletic cluster with *Mtb. hispanicum*, *Mtb. mesophilicum*, *Mtb. fujisawaense* and *Mtb. radiotolerans*,

Table 1. Differential characteristics of the strains characterized in this study and their closest phylogenetic neighbours

Strains: 1, *Mtb. persicinum* sp. nov. 002-165^T; 2, *Mtb. komagatae* sp. nov. 002-079^T; 3, *Mtb. mesophilicum* DSM 1708^T; 4, *Mtb. brachiatum* sp. nov. B0021^T; 5, *Mtb. brachiatum* sp. nov. Hojyo2; 6, *Mtb. brachiatum* sp. nov. RB603B; 7, *Mtb. tardum* sp. nov. RB677^T; 8, *Mtb. radiotolerans* IAM 12098^T; 9, *Mtb. fujisawaense* DSM 5686^T; 10, *Mtb. hispanicum* DSM 16372^T (data from Gallego *et al.*, 2005a); 11, *Mtb. hispanicum* DSM 1857; 12, *Mtb. gregans* sp. nov. 002-074^T; 13, *Mtb. gregans* sp. nov. RB678. +, Positive; –, negative; w, weak; tr, trace (<0.5 %; fatty acids that account for <0.5 % of the total are not shown); ND, not detected or no data.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Isolation source	Water	Water	Leaf surface	Water	Water	Water	Water	Rice grains	Unknown	Drinking water	Tap water	Water	Water
Colony diameter (mm)	0.5–0.6	1.1–1.3	0.4–1.4	0.8–1.6	1.1–1.6	0.5–2.2	0.7–0.8	ND	ND	1–2	1.0–1.2	1.1–1.4	0.9–1.2
Colony pigmentation	Light pink	Light pink to red	Whitish pink	Light pink	Pink	Pink	Pink	Pink to red ^{a*}	Pink ^b	Pink	Pink	Pink	Pink
Cell width (µm)	1.2–1.3	1.1–1.7	1.0–2.2	1.0–1.2	1.3–1.4	1.1–1.6	1.3–2.1	0.6–0.8 ^a	ND	1–1.5	1.2–1.5	1.3–1.4	1.4–1.5
Cell length (µm)	2.8–3.9	2.3–4.9	2.3–4.4	2.5–4.3	2.0–8.6	1.7–5.2	2.0–5.1	1.4–2.5 ^a	ND	2–2.5	1.9–4.5	2.1–4.0	2.3–5.2
Growth temperature (°C)													
Range	10–35	10–35	10–30	10–35	10–35	4 (w)–40 (w)	15–35	ND	15–37 ^b	15–30	10–35	15–35	10–35
Optimum	25–30	25	ND	30	30	30	30	20–32 ^a	ND	28	30	25	25
Oxidase	+	+	+	+	+	+	+	+	–	+	–	+	+
Utilization of carbon sources													
L-Arabinose	+	w	w	w	+	+	w	+	+	–	–	–	w
D-Xylose	w	–	w	+	+	+	–	+	w	–	w	–	+
D-Fucose	–	–	w	–	–	–	w	+	w	–	w	w	w
D-Fructose	–	+	–	–	–	–	–	–	–	+	+	+	+
D-Glucose	–	w	w	–	–	–	–	+	–	–	–	–	–
Betaine	–	–	–	–	–	–	–	+	–	–	–	–	–
Acetate	+	+	w	–	–	–	–	+	+	+	+	+	+
N,N-Dimethylformamide	–	–	–	–	–	–	–	–	–	–	w	–	–
L-Aspartate	–	–	–	–	–	–	–	+	+	–	–	–	+
Quinone composition (% of total)													
Ubiquinone Q-9	ND	6.5	3.7	3.4	4.4	ND	6.4	4.1	5.8	2.8	4.3	ND	ND
Ubiquinone Q-10	100.0	93.5	96.3	96.6	95.6	100.0	93.6	95.9	94.2	97.2	95.7	100.0	100.0
Fatty acid composition (% of total)													
C _{10:0} 3-OH	ND	ND	ND	ND	tr	ND	2.0	ND	tr	ND	ND	ND	tr
C _{15:0} anteiso	1.5	1.5	0.9	1.1	0.8	0.7	0.7	1.0	1.0	1.5	1.2	1.0	0.9
C _{16:0} iso	1.3	4.8	2.1	3.3	1.1	1.4	tr	1.1	1.9	3.9	2.8	1.7	1.9
C _{16:1} ω7c	6.1	2.9	5.3	3.6	3.8	5.0	4.8	5.1	3.6	2.2	2.6	1.6	2.1
C _{17:0} anteiso	ND	ND	tr	tr	ND	ND	ND	0.5	tr	ND	ND	ND	ND
C _{18:1} ω9c	ND	ND	ND	ND	0.9	0.8	0.8	ND	ND	ND	ND	ND	tr
C _{18:1} ω7c	75.8	82.6	85.6	87.2	83.8	79.2	77.9	87.8	88.5	82.9	83.7	86.9	85.9
C _{18:0}	14.1	7.1	4.3	3.2	7.8	11.4	11.8	3.4	3.3	8.2	8.3	7.8	7.6
Summed feature 6†	1.3	1.1	0.8	0.8	0.8	0.8	0.5	0.8	0.8	1.4	1.2	1.1	1.1
DNA G + C content (mol%)	67.1	68.7	69.2	69.7	69.2	69.6	69.5	70.1	70.9	71.5	71.1	69.9	70.1

*Data from other studies as follows: *a*, Ito & Iizuka (1971); *b*, Green *et al.* (1988).

†Summed feature 6 comprises C_{18:2} ω6,9c and/or anteiso-C_{18:0}.

which was supported by high bootstrap probabilities (87 %). Generally, the threshold of sequence similarity to separate species based on the 16S rRNA gene sequence is 97 % (Stackebrandt & Goebel, 1994), suggesting that the small phylogenetic distances of the new isolates from the most closely related species do not warrant their separation as members of different species. On the other hand, the *gyrB* gene sequence-based phylogenetic tree showed more highly

discriminative topology, and the eight strains were grouped monophyletically in a cluster separated from neighbouring species with 87.2–95.2 % similarity, clearly delineating them as members of distinct species. However, sequence similarity values used to separate species based on the *gyrB* gene vary depending on the genus. For instance, the threshold value of *gyrB* gene sequence divergence to separate *Oceanospirillum* species was concluded to be about 20 % (Satomi *et al.*, 2002),

Fig. 1. Phylogenetic neighbour-joining tree reconstruction using 16S rRNA gene sequences (1406 nt) showing the relationship between the eight isolated strains and members of the genus *Methylobacterium*. Only bootstrap values (expressed as percentage of 1000 replications) greater than 70% are shown at nodes. Solid circles indicate that the corresponding nodes are also recovered in maximum-parsimony trees. The sequence of *Rhodopseudomonas palustris* DSM 123^T was used as an outgroup. Bar, 1% sequence dissimilarity.

whereas the value for the genus *Shewanella* was defined as 10% (Venkateswaran *et al.*, 1999). Thus, to confirm the novelty of these isolates generated from the results of the phylogenetic analyses, DNA–DNA hybridization was performed.

DNA–DNA hybridization results (Table 2) revealed that the eight isolated strains could be assigned to five separate groups, distinct from previously described species. Strains 002-165^T and 002-079^T showed less than 10% DNA relatedness to the type strains of previously described

Table 2. DNA–DNA hybridization values between the novel isolates and strains of related *Methylobacterium* species

Values are mean percentages from three or more replicate experiments. Values for conspecific strains are highlighted in bold.

Strain	DNA–DNA hybridization (%) with:												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1. <i>Mtb. persicinum</i> sp. nov. 002-165 ^T	—	5	5	7	6	5	8	1	2	3	2	4	6
2. <i>Mtb. komagatae</i> sp. nov. 002-079 ^T	3	—	4	5	8	9	3	0	2	3	2	3	6
3. <i>Mtb. mesophilicum</i> DSM 1708 ^T	1	1	—	31	30	45	1	16	8	1	4	3	6
4. <i>Mtb. brachiatum</i> sp. nov. B0021 ^T	8	4	41	—	92	80	6	12	11	6	3	1	4
5. <i>Mtb. brachiatum</i> sp. nov. Hojyo2	1	1	41	79	—	87	0	12	10	7	2	0	2
6. <i>Mtb. brachiatum</i> sp. nov. RB603B	1	3	34	68	74	—	4	9	7	6	2	0	3
7. <i>Mtb. tardum</i> sp. nov. RB677 ^T	1	0	17	8	11	17	—	31	18	0	3	3	6
8. <i>Mtb. radiotolerans</i> IAM 12098 ^T	0	0	16	9	9	8	25	—	37	1	3	3	3
9. <i>Mtb. fujisawaense</i> DSM 5686 ^T	2	2	17	10	4	18	13	47	—	1	2	3	3
10. <i>Mtb. hispanicum</i> DSM 16372 ^T	3	3	11	0	2	5	0	1	3	—	72	33	43
11. <i>Mtb. hispanicum</i> DSM 1857	0	0	11	0	0	4	0	1	2	88	—	40	45
12. <i>Mtb. gregans</i> sp. nov. 002-074 ^T	1	1	10	4	2	4	0	2	3	36	48	—	88
13. <i>Mtb. gregans</i> sp. nov. RB678	1	0	9	2	2	4	0	2	2	33	42	87	—

The results from analyses of phenotypic characteristics and 16S rRNA and *gyrB* gene sequences and DNA hybridization tests strongly indicated that the eight novel strains represent five novel species within the genus *Methylobacterium*, although fatty acid methyl ester analysis and physiological tests failed to discriminate between neighbouring species. Moreover, the *gyrB* gene sequence similarity among neighbouring species (Table 1) and the *gyrB*-based phylogenetic tree (Fig. 2) clearly showed that *Methylobacterium chloromethanicum* and *Methylobacterium dichloromethanicum* were closely related to *Methylobacterium extorquens* and that *Methylobacterium lusitanum* was also related to *Mth. rhodesianum*. These results were supported by previously reported DNA–DNA hybridization data (Kato *et al.*, 2005),

On the basis of the data from the polyphasic studies described above, the eight isolates represent five novel species of the genus *Methylobacterium*, for which the names *Methylobacterium persicinum* sp. nov., *Methylobacterium komagatae* sp. nov., *Methylobacterium brachiatum* sp. nov., *Methylobacterium tardum* sp. nov. and *Methylobacterium gregans* sp. nov. are proposed. In addition, strain DSM 1857 was identified as a member of *Mtb. hispanicum*.

The transfer of *Mtb. chloromethanicum* and *Mtb. dichloromethanicum* strains to *Mtb. extorquens* and of *Mtb. lusitanum* strains to *Mtb. rhodesianum* has already been proposed by Kato *et al.* (2005, 2006). However, the opinion of reclassification cannot be considered as validly published nor, in any other way, approved by the International Committee on Systematics of Prokaryotes and its Judicial Commission. Therefore, we propose emended descriptions of *Methylobacterium extorquens* and *Methylobacterium rhodesianum*.

The description is based on the original descriptions of *Methylobacterium extorquens*, *Methylobacterium chloromethanicum* McDonald *et al.* 2001 and *Methylobacterium*

dichloromethanicum Doronina *et al.* 2000. The original description of the species *Methylobacterium* (= *Protomonas*) *extorquens* was published by Urakami & Komagata (1984). Strains CM4 and DM4, the type strains of *Methylobacterium chloromethanicum* and *Methylobacterium dichloromethanicum*, respectively, are reference strains of *Methylobacterium extorquens*. The type strain is strain TK 0001^T (=DSM 1337^T =NCIMB 9399^T).

Emended description of *Methylobacterium rhodesianum* Green *et al.* 1988

The description is based on the original descriptions of *Methylobacterium rhodesianum* and *Methylobacterium lusitanum* Doronina *et al.* 2002. The description of the species *Methylobacterium rhodesianum* was given by Green *et al.* (1988). Strain RXM, the type strain of *Methylobacterium lusitanum*, is a reference strain of *Methylobacterium rhodesianum*. The type strain is strain NCIMB 12249^T (=ATCC 43882^T =DSM 5687^T).

Description of *Methylobacterium persicinum* sp. nov.

Methylobacterium persicinum (per.si'ci.num. N.L. neut. adj. *persicinum* peach-coloured, because of its pink colour).

Cells are Gram-negative, motile, ovoid or slightly curved rods (1.2–1.3 × 2.8–3.9 µm) after 6 days culture on GP agar. Strictly aerobic. Colonies grown on GP agar for 9 days are circular and smooth with a diameter of approximately 0.5 mm and are light pink in colour. Growth occurs at 10–35 °C, but not at 4 or 40 °C. The optimum growth temperature is 25–30 °C. Neutrophilic. Oxidase- and catalase-positive. The ability to utilize methanol is weak and utilization occurs at 25–30 °C in the second week of cultivation. Substrate utilization, enzyme production, acid production and other physiological characteristics are given in Table 1. The respiratory quinone is ubiquinone Q-10 (100 %). Major cellular fatty acids are C_{18:1}ω7c (75.8 %), C_{18:0} (14.1 %), C_{16:1}ω7c (6.1 %) and C_{16:0} iso (1.3 %). The DNA G+C content of the type strain is 67.1 mol% (as determined by HPLC).

The type strain, 002-165^T (=DSM 19562^T =NBRC 103628^T =NCIMB 14378^T), was isolated from a water sample from a food factory in Japan.

Description of *Methylobacterium komagatae* sp. nov.

Methylobacterium komagatae (ko.ma.ga'tae. N.L. masc. gen. n. *komagatae* named after Dr Kazuo Komagata, in honour of his significant contributions to the study of facultatively methylophilic bacteria).

Cells are Gram-negative, motile, ovoid to straight or slightly curved rods (1.1–1.7 × 2.3–4.9 µm) after 6 days culture on GP agar. Strictly aerobic. Colonies grown on GP

agar for 9 days are circular and smooth with a diameter of approximately 1 mm and are light pink to red in colour. Growth occurs at 10–35 °C, but not at 4 or 40 °C. The optimum growth temperature is 25 °C. Neutrophilic. Oxidase- and catalase-positive. The ability to utilize methanol is weak and utilization occurs at 10–30 °C in the second week of cultivation. Substrate utilization, enzyme production, acid production and other physiological characteristics are given in Table 1. The major respiratory quinone is ubiquinone Q-10 (93.5 %); ubiquinone Q-9 (6.5 %) is also present. Major cellular fatty acids are C_{18:1}ω7c (82.6 %), C_{18:0} (7.1 %), C_{16:0} iso (4.8 %) and C_{16:1}ω7c (2.9 %). The DNA G+C content of the type strain is 68.7 mol% (as determined by HPLC).

The type strain, 002-079^T (=DSM 19563^T =NBRC 103627^T =NCIMB 14377^T), was isolated from a water sample in a food factory in Japan.

Description of *Methylobacterium brachiatum* sp. nov.

Methylobacterium brachiatum (bra.chi.a'tum. L. neut. adj. *brachiatum* branched, referring to the presence of branched cells).

Cells are Gram-negative, motile, straight to slightly curved or winding rods (1.0–1.2 × 2.5–4.3 µm) after 6 days culture on GP agar. At later stages of growth, cells are branched and pleomorphic. Strictly aerobic. Colonies grown on GP agar for 9 days are generally circular and smooth, although in some strains they have a rough, wrinkled and tough surface. Colonies are approximately 0.5–2.2 mm in diameter and light pink in colour. Growth occurs at 10–35 °C; some strains grow weakly at 4 and 40 °C. The optimum growth temperature is 30 °C. Neutrophilic. Oxidase- and catalase-positive. Substrate utilization, enzyme production, acid production and other physiological characteristics are given in Table 1. The major respiratory quinone is ubiquinone Q-10 (95.6–100 %); ubiquinone Q-9 (0–4.4 %) is present in some strains. Major cellular fatty acids are C_{18:1}ω7c (79.2–87.2 %), C_{18:0} (3.2–7.8 %), C_{16:1}ω7c (3.6–5.0 %) and C_{16:0} iso (1.1–3.0 %). The DNA G+C content is 69.2–69.7 mol% (as determined by HPLC).

The type strain, B0021^T (=DSM 19569^T =NBRC 103629^T =NCIMB 14379^T), and reference strains Hojoyo2 (=DSM 19568 =NBRC 103630) and RB603B (=DSM 19567 =NBRC 103631) were isolated from water samples from food factories in Japan.

Description of *Methylobacterium tardum* sp. nov.

Methylobacterium tardum (tar'dum. L. neut. adj. *tardum* slow, referring to the slow growth of the organism).

Cells are Gram-negative, motile, straight to slightly curved or winding rods (1.3–2.1 × 2.0–5.1 µm) after 6 days culture on GP agar. Strictly aerobic. Colonies grown on GP agar for 9 days are circular and smooth with a diameter of

approximately 0.7–0.8 mm and are pink in colour. Growth occurs at 15–35 °C, but not at 10 or 40 °C. The optimum growth temperature is 30 °C. Neutrophilic. Oxidase- and catalase-positive. The ability to utilize methanol is weak and utilization occurs at 25–35 °C in the second week of cultivation. Substrate utilization, enzyme production, acid production and other physiological characteristics are given in Table 1. The major respiratory quinone is ubiquinone Q-10 (93.6 %); ubiquinone Q-9 (6.4 %) is also present. Major cellular fatty acids are C_{18:1}ω7c (77.9 %), C_{18:0} (11.8 %), C_{16:1}ω7c (4.8 %) and C_{10:0} 3-OH (2.0 %). The DNA G + C content of the type strain is 69.5 mol% (as determined by HPLC).

The type strain, RB677^T (=DSM 19566^T =NBRC 103632^T =NCIMB 14380^T), was isolated from a water sample from a food factory in Japan.

Description of *Methylobacterium gregans* sp. nov.

Methylobacterium gregans (gre'gans. L. part. adj. *gregans* collecting into a flock or herd, referring to the floc formation in static media).

Cells are Gram-negative, motile, ovoid to straight or slightly curved rods (1.3–1.5 × 2.1–5.2 µm) after 6 days culture on GP agar. Strictly aerobic. Colonies grown on GP plate for 9 days are circular and smooth with a diameter of approximately 0.9–1.4 mm and are pink in colour. In static liquid media, a floc is formed. Growth occurs at 10–35 °C, but not at 4 or 40 °C. The optimum growth temperature is 25 °C. Neutrophilic. Oxidase- and catalase-positive. The ability to utilize L-arabinose, D-xylose and L-aspartate is variable. Other substrate utilization, enzyme production, acid production and other physiological characteristics are given in Table 1. The respiratory quinone is ubiquinone Q-10 (100 %). Major cellular fatty acids are C_{18:1}ω7c (85.9–86.9 %), C_{18:0} (7.6–7.8 %) and C_{16:1}ω7c (1.6–2.1 %). The DNA G + C content is 69.9–70.1 mol% (as determined by HPLC).

The type strain 002-074^T (=DSM 19564^T =NBRC 103626^T =NCIMB 14376^T) and reference strain RB678 (=DSM 19565 =NBRC 103633) were isolated from water samples from food factories in Japan.

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References

- Bousfield, I. J. & Green, P. N. (1985). Reclassification of bacteria of genus *Protomonas* Urakami and Komagata 1984 in the genus *Methylobacterium* (Patt, Cole, and Hanson) emend. Green and Bousfield 1983. *Int J Syst Bacteriol* **35**, 209.
- Doronina, N. V., Trostenko, Y. A., Tourova, T. P., Kuznetsov, B. B. & Leisinger, T. (2000). *Methylophilia helvetica* sp. nov. and *Methylobacterium dichloromethanicum* sp. nov. – novel aerobic facultatively methylotrophic bacteria utilizing dichloromethane. *Syst Appl Microbiol* **23**, 210–218.
- Doronina, N. V., Trostenko, 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**, 773–777.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2005a). *Methylobacterium hispanicum* sp. nov. and *Methylobacterium aquaticum* sp. nov., isolated from drinking water. *Int J Syst Evol Microbiol* **55**, 281–287.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2005b). *Methylobacterium variabile* sp. nov., a methylotrophic bacterium isolated from an aquatic environment. *Int J Syst Evol Microbiol* **55**, 1429–1433.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2005c). *Methylobacterium isbiliense* sp. nov., isolated from the drinking water system of Sevilla, Spain. *Int J Syst Evol Microbiol* **55**, 2333–2337.
- Gallego, V., Garcia, M. T. & Ventosa, A. (2006). *Methylobacterium adhaesivum* sp. nov., a methylotrophic bacterium isolated from drinking water. *Int J Syst Evol Microbiol* **56**, 339–342.
- Goto, K., Omura, T., Hara, Y. & Sadaie, Y. (2000). Application of the partial 16S rDNA sequence as an index for rapid identification of species in the genus *Bacillus*. *J Gen Appl Microbiol* **46**, 1–8.
- Gräf, W. & Bauer, L. (1973). Roter Bakterienaufwuchs (*Corynebacterium rubrum* n. spec.) in Leitungswassersystemen. *Zentralbl Bacteriol [Orig B]* **157**, 291–303 (in German).
- Green, P. (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-Verlag.
- 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.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Ito, H. & Iizuka, H. (1971). Taxonomic studies on a radio-resistant *Pseudomonas*. Part XII. Studies on the microorganisms of cereal grain. *Agric Biol Chem* **35**, 1566–1571.
- Kasai, H., Tamura, T. & Harayama, S. (2000). Intrageneric relationship among *Micromonospora* species deduced from *gyrB*-based phylogeny and DNA relatedness. *Int J Syst Evol Microbiol* **50**, 127–134.
- 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., Arai, D., Goto, K. & Yokota, A. (2006). *Methylobacterium chloromethanicum* pro synon. *Methylobacterium extorquens*; *Methylobacterium dichloromethanicum* pro synon. *Methylobacterium extorquens*; *Methylobacterium lusitanum* pro synon. *Methylobacterium rhodesianum*. In *Notification of Changes in*

Taxonomic Opinion Previously Published Outside the IJSEM, List of Changes in Taxonomic Opinion no. 4. *Int J Syst Evol Microbiol* **56**, 1463.

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

Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.

Patt, T. E., Cole, G. C. & Hanson, R. S. (1976). *Methylobacterium*, a new genus of facultatively methylotrophic bacteria. *Int J Syst Bacteriol* **26**, 226–229.

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

Sano, H., Sakai, M. & Nichijima, M. (1996). Application of MS to the search for products from marine bacteria. *J Mass Spectrom Soc Jpn* **44**, 377–391 (in Japanese).

Satomi, M., Kimura, B., Hamada, T., Harayama, S. & Fujii, T. (2002). Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and *gyrB* genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum jannaschii* and

Pseudomonas stanieri to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. *Int J Syst Evol Microbiol* **52**, 739–747.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Urakami, T. & Komagata, K. (1984). *Protomonas*, a new genus of facultatively methylotrophic bacteria. *Int J Syst Bacteriol* **34**, 188–201.

Venkateswaran, K., Moser, D. P., Dollhopf, M. E., Lies, D. P., Saffarini, D. A., MacGregor, B. J., Ringelberg, D. B., White, D. C., Nishijima, M. & other authors (1999). Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int J Syst Bacteriol* **49**, 705–724.

Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* **61**, 1104–1109.