NOTE

***Methylobacterium suomiense* sp. nov. and *Methylobacterium lusitanum* sp. nov., aerobic, pink-pigmented, facultatively methylotrophic bacteria**

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Two aerobic, pink-pigmented, facultatively methylotrophic bacteria, strains F20^T and RXM^T, are described taxonomically. On the basis of their phenotypic and genotypic properties, the isolates are proposed as novel species of the genus *Methylobacterium*, *Methylobacterium suomiense* sp. nov. (type strain F20^T = VKM B-2238^T = NCIMB 13778^T) and *Methylobacterium lusitanum* sp. nov. (type strain RXM^T = VKM B-2239^T = NCIMB 13779^T).

Keywords: methanol, methylamine, *Methylobacterium*

The genus *Methylobacterium* is a group of strictly aerobic, Gram-negative, rod-shaped, pink-pigmented, facultatively methylotrophic (PPFM) bacteria that utilize methanol and other reduced one-carbon compounds via the serine pathway (Green, 1992). The genus *Methylobacterium* now consists of 12 PPFM species, with *Methylobacterium organophilum* as the type species (Patt *et al.*, 1976; Green & Bousfield, 1983; Green *et al.*, 1988; Green, 1992; Urakami *et al.*, 1993; Wood *et al.*, 1998; Doronina *et al.*, 2000; McDonald *et al.*, 2001). They are chemotaxonomically quite similar, and phenotypic differences among the *Methylobacterium* species are found in only a limited number of properties, such as carbon- and energy-source utilization. Therefore, DNA–DNA similarity and 16S rDNA sequencing play a dominant role in the identification of novel *Methylobacterium* species (Hiraishi *et al.*, 1995; Wood *et al.*, 1998; Doronina *et al.*, 2000; McDonald *et al.*, 2001). Molecular taxonomy data have also shown that the genus *Methylobacterium* represents a line of descent in the α -2 subclass of the *Proteobacteria*. Here, we report the formal taxonomic description of two novel PPFM strains, F20^T and RXM^T.

Strain F20^T was isolated from soil samples collected in Finland. Strain RXM^T was isolated from a sewage station in Portugal and was preliminarily classified as

Methylobacterium sp. (Gírio & Attwood, 1991). All strains were grown on medium K (Doronina *et al.*, 1998). Analysis of cytological, physiological and metabolic properties, DNA base composition and 16S rDNA sequencing of the novel isolates were performed as described earlier (Doronina *et al.*, 1998, 2000). DNA–DNA hybridizations were carried out on nitrocellulose membrane filters (0.22 μ m) according to the method of Denhardt (1966) in the following incubation mixture: 0.15 M NaCl and 0.015 M trisodium citrate, pH 7.0, with 30% formamide at 60 °C for 24 h. Deoxy[1',2',5'-³H]CTP and an N5500 nick-translation kit (Amersham International) were used for labelling the probe DNA.

Table 1 summarizes the results of tests for carbon-source utilization by the novel strains and 12 known species of the genus *Methylobacterium*. Strain F20^T is most close to *Methylobacterium rhodesianum*, while strain RXM^T is similar to *Methylobacterium zatmanii*. Strains F20^T and RXM^T used the isocitrate lyase-negative (*icl*[−]) variant of the serine pathway, as confirmed by the presence of hydroxypyruvate reductase, serine-glyoxylate aminotransferase and malate lyase and by the absence of isocitrate lyase (*icl*[−]). The appropriate key enzymes of the ribulose monophosphate and bisphosphate pathways (hexulose-phosphate synthase and ribulose-bisphosphate carboxylase, respectively) were not detected.

The DNA of strains F20^T and RXM^T was extracted (Marmur, 1961) and the 16S rRNA genes were amplified and sequenced (Lane, 1991). They were

Abbreviation: PPFM pink-pigmented, facultatively methylotrophic.

The GenBank accession numbers for the 16S rRNA gene sequences of strains F20^T and RXM^T are respectively AY009404 and AY009403.

Table 1. Differential characteristics of the species of the genus *Methylobacterium*

Utilization of various compounds as sole sources of carbon and energy is shown. Strains are identified as: 1, F20^T; 2, RXM^T; 3, *Methylobacterium extorquens* NCIMB 9399^T; 4, *Methylobacterium organophilum* ATCC 27886^T; 5, *Methylobacterium rhodesianum* NCIMB 12249^T; 6, *Methylobacterium zatmanii* NCIMB 12243^T; 7, *Methylobacterium rhodinum* ATCC 14821^T; 8, *Methylobacterium radiotolerans* JCM 2831^T; 9, *Methylobacterium mesophilicum* ATCC 29983^T; 10, *Methylobacterium aminovorans* JCM 8240^T; 11, *Methylobacterium fujisawaense* NCIMB 12417^T; 12, *Methylobacterium thiocyanatum* DSM 11490^T; 13, *Methylobacterium chloromethanicum* NCIMB 13688^T; 14, *Methylobacterium dichloromethanicum* DSM 6343^T. +/–, Used/not used; ND, not determined.

Compound	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Methylamine	+	+	+	+	+	+	+	–	–	+	–	+	+	+
Trimethylamine	–	–	–	+	–	–	–	–	–	+	–	–	–	–
Acetate	+	+	+	+	+	+	+	+	–	+	+	+	+	+
Citrate	–	–	–	–	–	–	+	+	+	–	+	+	+	+
L-Glutamate	–	–	+	+	–	–	+	+	+	+	+	+	+	+
D-Glucose	+	–	–	–	–	–	+	+	+	–	+	+	–	–
D-Xylose	–	–	–	–	–	–	+	+	+	–	+	+	–	–
Fructose	+	+	–	+	+	+	+	–	–	+	–	+	–	+
Betaine	+	–	+	–	+	–	+	+	–	+	–	ND	+	+

Fig. 1. Phylogenetic analysis of the 16S rRNA sequences of strains F20^T and RXM^T and other representatives of the genus *Methylobacterium* and related genera. Bootstrap values (expressed as percentages of 100 replicates) are shown at branch points; values greater than 85% were considered significant. Bar, Jukes–Cantor distance (5 nucleotide substitutions per 100 nucleotides) (Jukes & Cantor, 1969).

aligned using the CLUSTAL program for sequence alignment with representative organisms from the same and related genera of bacteria. Phylogenetic relationships were determined by the neighbour-joining methods (Saitou & Nei, 1987), with bootstrap analysis of 100 trees using the programs of the TREECON package (Van de Peer & De Wachter, 1994). A total of 1400 nucleotides were determined for the 16S rDNA of both strains, corresponding to positions 11–1485 (*Escherichia coli* numbering). Phylogenetic analysis (Fig. 1) showed them to be located within the genus *Methylobacterium*, as supported by high bootstrap values (100%), and they are most closely related to *Methylobacterium extorquens* (98.25–98.8% similarity).

ty) and *M. rhodesianum* (98.1–98.6% similarity). The level of sequence identity between strains F20^T and RXM^T was 97.5%. This level corresponds to inter-species separation for the genus *Methylobacterium* (94.2–99.4%). However, the two strains are distinct by rather low levels of DNA relatedness from each other (19%) and also from the known members of the genus *Methylobacterium* (15–50%) (Table 2). Based on DNA–DNA hybridization values (not more than 50%) and 16S rDNA sequence similarities, the strains under study may be regarded as novel *Methylobacterium* species, with the names *Methylobacterium suomiense* sp. nov. and *Methylobacterium lusitanum* sp. nov.

Table 2. DNA–DNA hybridization between the novel strains and representatives of the genus *Methylobacterium*

DNA–DNA hybridization was carried out according to the method of Denhardt (1966).

Strain	DNA–DNA hybridization (%) with	
	F20 ^T	RXM ^T
F20 ^T	100	18
RXM ^T	19	100
<i>M. extorquens</i> NCIMB 9399 ^T (= JCM 2802 ^T)	17	27
<i>M. organophilum</i> NCIMB 11278 ^T (= ATCC 27886 ^T)	21	18
<i>M. rhodesianum</i> JCM 2810 ^T (= NCIMB 12249 ^T)	27	26
<i>M. zatmanii</i> JCM 2819 ^T (= NCIMB 12243 ^T)	26	25
<i>M. rhodinum</i> JCM 2811 ^T (= ATCC 14821 ^T)	29	19
<i>M. radiotolerans</i> JCM 2831 ^T (= DSM 1819 ^T)	31	22
<i>M. mesophilicum</i> JCM 2829 ^T (= ATCC 29983 ^T)	34	17
<i>M. aminovorans</i> JCM 8240 ^T (= ATCC 51358 ^T)	23	50
<i>M. fujisawaense</i> NCIMB 12417 ^T	16	21
<i>M. thiocyanatum</i> DSM 11490 ^T	17	30
<i>M. chloromethanicum</i> VKM B-2223 ^T (= NCIMB 13688 ^T)	15	34
<i>M. dichloromethanicum</i> VKM B-2191 ^T (= DSM 6343 ^T)	22	27

Description of *Methylobacterium suomiense* sp. nov.

Methylobacterium suomiense (su.o.mi'en.se. N.L. neut. adj. *suomiense* of Suomi, the Finnish word for Finland).

Gram-negative, asporogenous rods, 0.8–1.0 × 1.2–2.5 µm, occurring singly or rarely in pairs. Cells are motile by a single polar flagellum. Grows slowly on nutrient agar and PYG agar. Colonies on methanol/salt agar are shiny, smooth, raised, entire, pink and 0.5–1 mm in diameter after 3 days growth at 30 °C. The pink pigment is water insoluble and has absorption maxima at 473, 499 and 532 nm in a mixture of chloroform and methanol (1:1). Nitrate is reduced to nitrite. Methyl red and Voges–Proskauer tests are negative. Produces indole from tryptophan on medium with nitrate as a nitrogen source. Does not hydrolyse gelatin or starch. Does not form ammonia or hydrogen sulfide. Produces acid oxidatively from D-fructose and D-glucose. Catalase-, lipase- and urease-positive. Oxidase activity is weak. Strict aerobe. Chemo-organotroph and facultative methylotroph, assimilating C₁ compounds by the *icl*[−] serine pathway. Utilized carbon sources are methanol, methylamine, ethanol, glycerol, D-glucose, D-fructose, galactose, acetate, succinate, malate, fumarate, pyruvate, α-ketoglutarate, betaine, N,N-dimethylglycine and sarcosine. The following are not utilized: L-arabinose, D-xylose, mannose, lactose, maltose, trehalose, raffinose, rhamnose, ribose, sucrose, sorbose, sorbitol, mannitol, inositol, soluble starch, tartrate, propionate, citrate, acetamide, formamide, dimethylformamide, serine, glycine, valine, arginine, aspartate, glutamate, mono- and dichloromethane, di- and trimethylamine, CO₂ + H₂ and methane. Growth factors are not required. Ammonium salts, nitrate,

methylamine, aspartate and glutamate are utilized as nitrogen sources. Optimal growth between pH 6.9 and 7.5 and at 25–30 °C. No growth occurs in the presence of 3% NaCl. The main cellular fatty acid is *cis*-vaccenic acid (C_{18:1}ω7; 84.7%). The major ubiquinone is Q-10. Dominant phospholipids are cardiolipin, phosphatidylethanolamine and phosphatidylcholine. The G + C content of the DNA is 65.8 mol% (T_m). The GenBank accession number for the 16S rRNA gene sequence of strain F20^T is AY009404.

The type strain, F20^T, was isolated from Finnish forest soil near Lammi Biological Station as a methanol-utilizing bacterium and has been deposited in the Russian Collection of Microorganisms as VKM B-2238^T, in the National Collections of Industrial, Food and Marine Bacteria as NCIMB 13778^T and in the DSMZ as DSM 14458^T.

Description of *Methylobacterium lusitanum* sp. nov.

Methylobacterium lusitanum (lu.si.ta'num. L. neut. adj. *lusitanum* of *Lusitania*, the Latin name of Portugal).

Gram-negative, asporogenous rods, 1.0–1.2 × 1.7–2.0 µm, occurring singly or rarely in pairs. Cells are motile by a single or two to three polar flagella. Slow growth on nutrient agar and PYG agar. Colonies on methanol/salt agar are shiny, smooth, raised, entire and pink, 1 mm in diameter after 3 days growth at 30 °C. The pink pigment is water insoluble and has absorption maxima at 473, 499 and 532 nm in a mixture of chloroform and methanol (1:1). Nitrate is reduced to nitrite. Methyl red and Voges–Proskauer tests are negative. Produces indole from tryptophan on medium with nitrate as nitrogen source. Does not hydrolyse

gelatin or starch. Does not form ammonia or hydrogen sulfide. Produces acid oxidatively from D-fructose. Catalase-, oxidase-, lipase- and urease-positive. Oxidase activity is weak. Strict aerobe. Chemo-organotroph and facultative methylotroph, assimilating C₁ compounds by the *icl*⁻ serine pathway. Utilized carbon sources are: methanol, methylamine, ethanol, glycerol, inositol, D-fructose, acetate, acetamide, succinate, malate, fumarate, pyruvate, α -ketoglutarate and propionate. The following are not utilized: L-arabinose, D-glucose, galactose, D-xylose, mannose, lactose, maltose, trehalose, raffinose, rhamnose, ribose, sucrose, sorbose, sorbitol, mannitol, inositol, soluble starch, tartrate, citrate, alanine, valine, glycine, arginine, aspartate, betaine, *N,N*-dimethylglycine, sarcosine, glutamate, mono- and dichloromethane, di- and trimethylamine, CO₂ + H₂ and methane. Growth factors are not required. Ammonium salts, nitrate, urea, aspartate, glutamate and alanine are utilized as nitrogen sources. Optimal growth between pH 6.9 and 7.5 and at 30–37 °C. No growth occurs in the presence of 3% NaCl. The main cellular fatty acid is *cis*-vaccenic acid (C_{18:1} ω 7; 83.1%). The major ubiquinone is Q-10. Dominant phospholipids are phosphatidylethanolamine and phosphatidylcholine. The G+C content of the DNA is 66.5 mol% (*T*_m).

The type strain, RXM^T, was isolated from a sewage station in Portugal as a methanol-utilizing bacterium and has been deposited in the Russian Collection of Microorganisms as VKM B-2239^T, in the National Collections of Industrial, Food and Marine Bacteria as NCIMB 13779^T and in the DSMZ as DSM 14457^T.

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