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Methylobacterium soli sp. nov. a methanol-utilizing bacterium isolated from the forest soil

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Abstract A Gram-negative, pink-pigmented, non-spore-forming rod shaped, methanol-utilizing bacterium, strain YIM 48816^T, was isolated from forest soil collected from Sichuan province, China. Strain YIM 48816^T can grow at 4–37°C, pH 5.0–7.0 and 0% NaCl (w/v). Based on 16S rRNA gene sequence similarity studies, it belonged to the genus *Methylobacterium*, and formed a phyletic line. The 16S rRNA gene sequence similarities were 96.2% to *Methylobacterium mesophilicum* DSM 1708^T and 96.0% to *Methylobacterium brachiatum* DSM 19569^T, and the phylogenetic similarities to all other *Methylobacterium* species with validly published names were less than 96.0%. The major menaquinones detected were Q-10 (97.14%) and Q-9 (2.86%). The major fatty acids were C18:1 ω7c (80.84%). The DNA G + C content was 66.2 mol%. It is apparent from the genotypic and phenotypic data that strain YIM 48816^T belongs to a novel species of the genus *Methylobacterium*, for which the name *Methylobacterium soli* sp. nov. is proposed. The type strain is YIM 48816^T (CCTCC AA 208027^T = KCTC 22810^T).

Keywords *Methylobacterium soli* sp. nov · Soil · 16S rRNA gene

The GenBank accession number for the 16S rRNA gene sequence of strain YIM 48816^T is EU860984.

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Introduction

The genus *Methylobacterium* was established by Patt et al. (1976). The species of this genus exist in many kinds of habitation such as solid, water and air. Growing on one-carbon and multicarbon compounds is the characteristic of this genus. So far, the genus *Methylobacterium* comprised 34 validly described species (http://www.bacterio.cict.fr/m/methylobacterium.html).

Materials and methods

Isolation and maintenance of organism

During the course of studying the relationship between bacterial diversity and the various altitudes

of the mountain, we got the strain YIM 48816^T from the soil which altitude was 3300 m, collecting from Sichuan, Southwest of China. 2 g of the soil sample (dried for 7 days at room temperature and dry heated for 1 h at 80°C) was suspended in 20 ml sterilized distilled water, then shaken at 180 rpm for 1 h. The solution was spreaded onto ISP media 5 (Shirling and Gottlieb 1966). On the basis of colony morphology, one pink-pigmented strain (YIM 48816^T) were purified and maintained on the medium ISP 2 (Shirling and Gottlieb 1966). The strain was highly odoriferous.

Molecular analysis

Biomass for molecular systematic studies was derived from a medium ISP 2 shake culture incubated at 28°C for 10 days, harvested by centrifugation and washed twice using distilled water. Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene from the species YIM 48816^T were carried out by established procedures (Li et al. 2007). The 16S rRNA gene sequences of related taxa were obtained from the EzTaxon server (http://www.eztaxon.org/) (Chun et al. 2007). Multiple alignments of sequence similarity were carried out using CLUSTAL_X (Thompson et al. 1997). Phylogenetic analyses were inferred using three tree-making algorithms, the neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony (Fitch 1971) methods. The tree topology was performed by the neighbour-joining method using MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar et al. 2004), and Kimura two-parameter model (Kimura 1980) was used to calculate the distances. Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data based on 1000 resamplings (Felsenstein 1985).

Because strain YIM 48816^T showed low levels of 16S rRNA gene sequence similarities with all validly published species of the genus *Methylobacterium*, it was subjected to a polyphasic taxonomic investigation.

Morphological, physiological and biochemical characteristics

The Gram reaction was performed by using the standard Gram reaction and confirmed by using the KOH lysis test method (Cerny 1978). Morphological characteristics were observed by light microscopy (BH2 microscope; Olympus) after 6 days growth on ISP 2 agar medium at 28°C. Salt tolerance was tested in ISP 2 medium supplemented with 1–10% (w/v) NaCl. The effects of temperature on growth were examined at 0, 4, 10, 15, 20, 28, 37, 45 and 55°C for 14 days. Growth at different pH concentrations (from 4.0 to 10.0 at intervals of 1 pH unit) was assessed after incubation for 2 weeks as described by Xu et al. (2005). Carbon and nitrogen source utilization was tested as described by Gordon et al. (1974). Catalase activity was determined by production of bubbles after the addition of a drop of 3% H_2O_2. Oxidase activity was observed by oxidation of tetramethyl-p-phenylenediamine. Urease activity, H_2S production, nitrate reduction, milk coagulation and peptonization, hydrolysis of gelatin, cellulose, starch and Tweens 20, 40, 60 and 80 were tested as described by Cowan and Steel (1965). Other enzyme activities were determined by using the API ZYM systems (bioMe′rieux) according to the manufacturer's instructions.

Chemotaxonomy

To measure the G + C content of the DNA, it was enzymically degraded into nucleosides and investigated as described by Mesbah et al. (1989), using reversed-phase HPLC. Chemosystematic studies were carried out to establish whether isolate YIM 48816^T had a chemical profile consistent with its assignment to the genus *Methylobacterium*. Cell biomass for quinone analysis was obtained from cultivation in ISP 2 medium shake culture incubated at 28°C for 10 days. The isoprenoid quinone was investigated as described by Komagata and Suzuki (1987), using reversed-phase HPLC. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI), and identified using the Microbial Identification software package (Sasser 1990).

Results and discussion

The 16S rRNA gene sequence of the strain YIM 48816^T was a continuous stretch of 1405 bp. Sequence similarity calculations and phylogenetic

analysis revealed that strain YIM 48816^T was closely related to *M. mesophilicum* DSM 1708^T (Austin and Goodfellow 1979; Green and Bousfield 1983) and *M. brachiatum* DSM 19569^T (Kato et al. 2008), with sequence similarities of 96.2 and 96.0%, respectively. The similarities with respect to all other members of this genus were below 96.0%. A phylogenetic tree was constructed with sequences of representative strains of the genus *Methylobacterium*, and the type strain of *Balneimonas flocculans* TFB^T was used as the outgroup. The phylogenetic tree (Fig. 1) based on the neighbour-joining algorithm showed that strain YIM 48816^T falls within the genus *Methylobacterium*, forming a distinct phyletic line with a high bootstrap resampling value of 72%, which confirmed that the new isolate belonged to *Methylobacterium,* but differentiated from all the recognized species of the genus *Methylobacterium*.

The isolate YIM 48816^T was determined as comprising Gram-negative, and formed pink- to light red-pigmented colonies. Cells of the strain were rods and no spores were observed. Cells of the strain often contained poly-β-hydroxybutyrate or volutin granules (Fig. 2). Strain YIM 48816^T did not grow in the presence of 1% NaCl, it was able to grow at 4–37°C and at pH 5.0–7.0. The optimum growth occurred at 28°C and pH 7.0 on ISP 2 agar medium. Cells were oxidase- and catalase-positive. The other physiological properties determined for strain YIM 48816^T are given in Table 1 and in the species description.

The genomic DNA G + C content of strain YIM 48816^T was 66.2 mol%. The predominant ubiquinone was Q-10. The primary fatty acid was $C_{18:1}$ ω7c (88.46%). All of these chemical properties are consistent with the classification of the isolate YIM 48816^T in the genus *Methylobacterium*.

Taxonomic conclusion

The 16S rRNA gene sequence, phylogenetic data showed that the isolate YIM 48816^T was a member of the genus *Methylobacterium*. Also, the following characteristics (cell shape, the predominant ubiquinone and some other physiological properties) of strain YIM 48816^T are consistent with its assignment to the genus *Methylobacterium*. Whereas, the low levels of 16S rRNA gene sequence similarity distinguish the novel isolate from its closest neighbours in the genus *Methylobacterium*. Table 1 provides a comparison of the characteristics of strain YIM 48816^T and closely related species of the genus

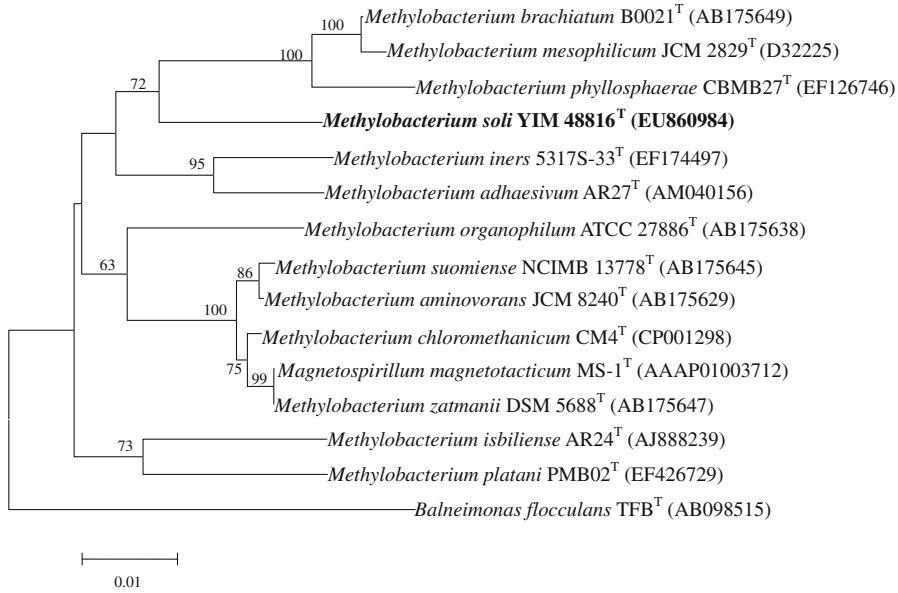

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of isolate YIM 48816^T within the *Methylobacterium* genus. Numbers at nodes are bootstrap percentages (based on 1000 resampled datasets); only values above 50% are given. The sequence of *Balneimonas flocculans* TFB^T (GenBank accession no. AB098515) was used as an outgroup. *Bar* 0.01 substitutions per nucleotide position

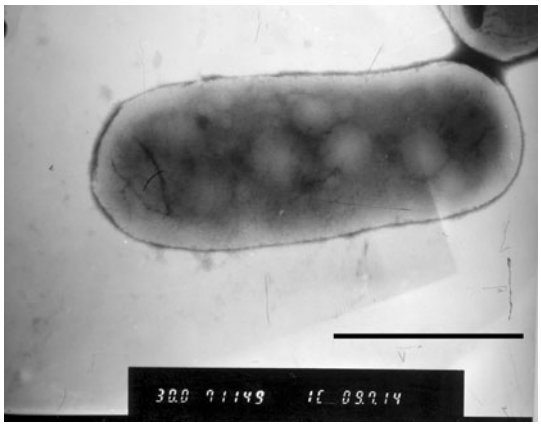

Fig. 2 Transmission electron micrograph showing the general cell morphology of strain YIM 48816^T and the presence of the poly-β-hydroxybutyrate or volutin granules after growth on ISP 2 agar for 6 days at 28°C. *Bar* 1 μm

Methylobacterium. The strain YIM 48816^T can use D-fructose, *N,N*-dimethylformamide and L-aspartate as carbon sources, but not L-arabinose or D-xylose; the closest strains *M. mesophilicum* DSM 1708^T and *M. brachiatum* DSM 19569^T yielded opposite results. Additionally, the fatty acids of *M. mesophilicum* and *M. brachiatum* have traces of C18 :1ω9c, which is absent in the strain YIM 48816^T. DNA G + C content of strain YIM 48816^T was lower 3% than the closest strains *M. mesophilicum* DSM 1708^T and *M. brachiatum* DSM 19569^T. These physiological and biochemical characteristics (Table 1) evidently support the strain YIM 48816^T represents a novel species of the genus *Methylobacterium*, for which the name *Methylobacterium soli* sp. nov. is proposed.

Description of *Methylobacterium soli* sp. nov

Methylobacterium soli (so'li. L. neut. gen. n. soli of soil, the source of the organism). Cells are Gram-negative, non-spore-forming, rod-shaped (0.7–1.0 × 1.4–2.5 μm), containing poly-β-hydroxybutyrate or

Table 1 Differential characteristics between strain YIM 48816^T and their closest phylogenetic neighbours of the genus *Methylobacterium*.

Characteristic	1	2[a]	3[a]
Isolation source	Soil	Water	Leaf
Cell width (μm)	0.7–1.1	1.0–2.2	1.1–1.6
Cell length (μm)	1.4–2.5	2.3–4.4	1.7–5.1
Growth temperature (°C) Range	4(w)–37(w)	10–37(w)	4(w)–40
Carbon source			
L-Arabinose	−	w	+
D-Xylose	−	+	+
D-Fucose	+	w	−
D-Fructose	+	−	w
D-Glucose	+	w	w
Acetate	−	w	−
N,N-Dimethylformamide	w	−	−
L-Aspartate	+	−	−
Quinone composition (% of total)			
Ubiquinone Q-9	2.9	3.7	0
Ubiquinone Q-10	97.1	96.3	100.0
Fatty acid composition (% of total)			
C18 : 1ω7c	88.46	81.99	87.77
C18 : 1ω9c	0	1.10	0.57
C18 : 0	3.36	5.51	3.62
C16 : 0	3.29	8.32	3.99
C14 : 0	0.54	0.76	0.52
Sum In Feature 2	1.72	0.65	0.78
Sum In Feature 3	2.12	1.66	2.05
DNA G + C content (mol%)	66.2	69.2	69.6

Strains: *1* strain YIM 48816^T, *2 M. mesophilicum* DSM 1708^T, *3 M. brachiatum* DSM 19569^T. + Positive, − negative, *w* weak

[a] Data of the strains are from the parallel experiments

volutin granules. Colonies are pink to light red-coloured, circular and smooth with non-pigmented after growth on ISP 2 agar for 6 days at 28°C. Catalase- and oxidase-positive, but negative for urease activity, H_2S production, nitrate reduction, milk coagulation and peptonization, hydrolysis of gelatin, cellulose, starch and Tweens 20, 40, 60 and 80 tests. Growth is observed at 4–37°C (optimum 28°C) and pH 5.0–7.0 (optimum 7.0). No growth in the presence of NaCl concentrations of 1% or more. Enzyme activities (tested in the API ZYM system) are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, negative for α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase. Carbon sources utilized include D-glucose, maltose, sucrose, D-fucose, lactose, D-raffinose, D-fructose, ribose, sorbitol, *myo*-inositol, D-mannitol, dextrin, methanol, acetate, *N,N*-dimethylformamide, L-aspartate. Does not utilize L-arabinose, cellobiose, D-galactose, L-rhamnose, mannose, D-galactose, D-xylose, glycerol, calcium DL-malate. Nitrogen sources utilized include L-histidine, DL-methionine, L-leucine, L-ornithine, L-alanine, L-proline, serine, tryptophan, phenylalanine, L-valine. Does not utilize xanthine, L-arginine, urea, lysine. Ubiquinone Q-10 (97.14%) is the predominant isoprenoid quinine, the other 2.86% content is Q-9. The major fatty acid is C18:1 ω7c (88.46%).

The type strain is YIM 48816^T (=CCTCC AA 208027^T = KCTC 22810^T), isolated from a forest soil sample in Sichuan province, Southwest of China. The DNA G + C content of the type strain is 66.2 mol%.

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