Methylobacterium adhaesivum sp. nov., a methylotrophic bacterium isolated from drinking water

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A pink-pigmented, facultatively methylotrophic strain, AR27^T, isolated from the drinking water distribution system of Seville, Spain, was characterized taxonomically. Cells were Gram-negative rods, motile, non-spore-forming and strictly aerobic. Growth in liquid media was flocculant whereas on solid media growth produced colonies that usually adhered to the agar surface. On the basis of its phenotypic characteristics, 16S rRNA gene sequence data and DNA G + C content (63.6 mol%), strain AR27^T (=CECT 7069^T = CCM 7305^T) is proposed as the type strain of a novel species of the genus *Methylobacterium*, *Methylobacterium adhaesivum* sp. nov.

The genus *Methylobacterium* belongs to the α -2 subclass of the *Proteobacteria* and has the serine pathway for formaldehyde assimilation. It includes strictly aerobic, Gram-negative, rod-shaped, pink-pigmented, facultatively methylotrophic (PPFM) bacteria, which can grow on single carbon compounds such as formate, formaldehyde and methanol as the sole source of carbon and energy, as well as on a wide range of multi-carbon growth substrates (Green, 2001). At the time of writing, the genus comprises 20 recognized species (Patt *et al.*, 1976; Green & Bousfield, 1983; Bousfield & Green, 1985; Green *et al.*, 1988; Urakami *et al.*, 1993; Wood *et al.*, 1998; Doronina *et al.*, 2000, 2002; McDonald *et al.*, 2001; Van Aken *et al.*, 2004; Jourand *et al.*, 2004; Gallego *et al.*, 2005a, b, c), with *Methylobacterium organophilum* as the type species (Patt *et al.*, 1976).

Members of the genus *Methylobacterium* are ubiquitous in terrestrial habitats (Green & Bousfield, 1981, 1983), including soil, dust, freshwater, tap water systems, lake sediments, leaf surfaces and nodules, rice grains and air, and as contaminants in various products and processes. The PPFM bacteria are strict aerobes and can be isolated from almost any freshwater environment where some dissolved oxygen exists, e.g. from tap water systems (Gräf & Bauer, 1973) and from stratified lake systems (Hanson, 1980). Furthermore, species of this genus have been isolated from hospital environments (Gilardi & Faur, 1984), where they may pose a threat as opportunistic pathogens to seriously ill patients.

A sampling campaign in April 2004 to identify culturable and non-culturable bacteria present in the drinking water distribution system of Seville, Spain, led to isolation of a slowly growing pink-pigmented bacterium. This isolate was studied phenotypically, phylogenetically and genotypically. Based on these characteristics we suggest that strain AR27^T represents a novel species of the genus *Methylobacterium*.

Drinking water samples (25 l) were concentrated by using a tangential flow filtration system (Filtron), plated on plate count agar (PCA; Difco) and R2A (Difco) and incubated at 28 °C for 7 days. Distinct colonies were picked in order to obtain pure cultures. Pink-pigmented colonies were selected and studied in more detail. One of these strains, AR27^T, showed a characteristic growth that differentiated it from the remaining pink-pigmented colonies isolated. Strain AR27^T was characterized phenotypically by using the methods described by Doronina *et al.* (1998). The use of different carbon compounds was determined as described previously by Gallego *et al.* (2005a) by using Biolog Microplates (Biolog Inc.).

Chromosomal DNA was isolated and purified according to the methods described by Wilson (1997) and a partially modified Marmur method (Marmur, 1961; Hood *et al.*, 1987). The 16S rRNA gene was amplified using the universal primers 16F27 and 16R1488 as described by Mellado *et al.* (1995). Sequencing was performed by NBT-Newbiotechnic (Seville, Spain) using an automated DNA sequencer model 3100 (Applied Biosystems), and an almost-complete nucleotide sequence was determined. Alignment of the 16S rRNA gene sequence was carried out by using the ARB program (<http://www.arb-home.de>). Base-frequency filters were applied in the sequence comparison analysis. Phylogenetic trees were inferred by using maximum-parsimony,

Published online ahead of print on 7 October 2005 as DOI 10.1099/ijls.0.63966-0.

Abbreviation: PPFM, pink-pigmented, facultatively methylotrophic.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain AR27^T is AM040156.

neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood methods. The G + C content of the genomic DNA was determined from the mid-point value (T_m) of the thermal denaturation profile (Marmur & Doty, 1962) by using the equation of Owen & Hill (1979).

Cells of strain AR27^T were Gram-negative rods, strictly aerobic, measuring 1·0–1·2 µm wide by 1·5–5·0 µm long after 24 h growth at 28 °C. Cells were motile. Colonies of strain AR27^T were circular, pink pigmented with a diameter of 0·5–0·8 mm when grown on R2A agar after 7 days incubation. Growth in liquid media was flocculant whereas on solid media growth produced colonies that usually adhered to the agar surface. Strain AR27^T was slow-growing, the doubling time typically being 48–72 h under optimal conditions. Growth did not occur in the presence of 1 % NaCl. Differential phenotypic characteristics between strain AR27^T and related *Methylobacterium* species are summarized in Table 1.

The almost-complete 16S rRNA gene (approximately 1400 bp) of strain AR27^T was sequenced directly following PCR amplification. Sequence similarity with other *Methylobacterium* species was ≤96·4 %, with *Methylobacterium fujisawaense* the most similar species. Moreover, the 16S

rRNA gene sequence phylogenetic analysis clearly showed that strain AR27^T was located in a branch separate from the remaining species of the genus (Fig. 1). According to the phylogenetic data the isolate belongs to the genus *Methylobacterium* but as it only shows a relatively low similarity with other species, strain AR27^T could be considered to represent a novel species.

The DNA G + C content of strain AR27^T was 63·9 mol%, the lowest value reported for strains of the genus *Methylobacterium* (Hood *et al.*, 1987; Urakami *et al.*, 1993; Green, 2001).

On the basis of these molecular and physiological characteristics, we propose to place strain AR27^T in a novel species, *Methylobacterium adhaesivum* sp. nov.

Description of *Methylobacterium adhaesivum* sp. nov.

Methylobacterium adhaesivum (ad.hae'si.vum. L. neut. adj. *adhaesivum* adhering, forming aggregates).

Cells are Gram-negative rods, 1·0–1·2 × 1·5–5·0 µm, occurring singly or forming aggregates (when grown in liquid R2A medium at 28 °C after 24 h). Cells are motile,

Table 1. Differential phenotypic characteristics of *Methylobacterium adhaesivum* sp. nov. and other related species of the genus *Methylobacterium*

Strains: 1, *Mtb. adhaesivum* AR27^T; 2, *Mtb. isbiliense* AR24^T; 3, *Mtb. variabile* GR3^T (Gallego *et al.*, 2005c); 4, *Mtb. hispanicum* CCM 7219^T (Gallego *et al.*, 2005a); 5, *Mtb. aquaticum* CCM 7218^T (Gallego *et al.*, 2005a); 6, *Mtb. aminovorans* JCM 8240^T (Urakami *et al.*, 1993); 7, *Mtb. suomiense* NCIMB 13778^T (Doronina *et al.*, 2002); 8, *Mtb. lusitanum* NCIMB 13779^T (Doronina *et al.*, 2002); 9, *Mtb. thiocyanatum* NCIMB 13651^T (Wood *et al.*, 1998); 10, *Mtb. chloromethanicum* NCIMB 13688^T (McDonald *et al.*, 2001); 11, *Mtb. populi* NCIMB 13946^T (Van Aken *et al.*, 2004); 12, *Mtb. nodulans* LMG 21967^T (Jourand *et al.*, 2004); 13, *Mtb. dichloromethanicum* DSM 6343^T (Doronina *et al.*, 2000). ND, Not determined; w, weak; v, variable; +, positive; –, negative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Cells occur:													
Singly	+	+	+	+	+	+	+	+	ND	ND	+	+	+
In pairs	Rarely	Rarely	+	Rarely	+	Rarely	Rarely	Rarely	ND	ND	+	Rarely	Rarely
Aggregates/rosettes	+	+	+	–	+	–	–	–	ND	ND	+	–	–
Cell length (µm)	1·5–5	2–5	2–6	2–2·5	4·5–8	1·5–4	1·2–2·5	1·7–2	2	2·5–3·5	1–10	1–1·5	1·0–2·0
Cell width (µm)	1–1·2	1–1·3	1–1·5	1–1·5	1·5–1·7	0·8–1	0·8–1	1–1·2	0·8	0·8–1	0·8–1	0·8–1·0	0·8–1·0
Pigmentation	Pink	Pink	Pink	Light pink	Pink to red	Pink or red	Pink	Pink	Pink	Pink	Pink	Not pigmented	Pink
Diameter of colonies (mm)	0·5–0·8	1–2	2–7	1–2	1–2	1–3	0·5–1	1	1·5	ND	0·1–0·2	0·5–1	1–3
Oxidase	+	+	–	+	–	+	+	+	v	ND	+	+	+
Growth on:													
Citrate	+	+	+	+	+	–	–	–	+	+	–	+	–
L-Glutamate	+	+	+	+	+	+	–	–	+	+	–	+	+
D-Glucose	–	–	+	–	+	–	+	–	+	–	–	–	–
D-Xylose	–	–	–	–	–	–	–	–	+	–	–	+	–
Fructose	+	–	+	+	+	+	+	+	+	–	+	–	+
DNA G + C content (mol%)	63·6	69	69·2	67·7	67·5	68	65·8	66·5	69·8–71	64·4	70·4	ND	67·1

Fig. 1. Phylogenetic tree showing the relationships of *Methylobacterium adhaesivum* AR27^T and species belonging to the genus *Methylobacterium* and other related methylotrophic bacteria. The tree was constructed using the maximum-parsimony method. Bootstrap values > 85% are indicated at branch points. Bar, 2% sequence divergence.

non-spore-forming and strictly aerobic. Growth in liquid media is flocculant whereas growth on solid media produces colonies that usually adhere to the agar surface. Colonies are pink and circular, 0.5–0.8 mm in diameter after 7 days at 28 °C on R2A agar. Growth is slow, doubling typically requiring 48–72 h under optimal conditions. No growth in the presence of $\geq 1.0\%$ NaCl. Growth occurs at 15–35 °C (optimum 28 °C) and at pH 5.0–9.0 (optimum pH 6.5). Catalase- and urease-positive. Oxidase activity is weak. Indole, methyl red and Voges–Proskauer reactions are negative. Tween, starch, gelatin, casein and DNA are not hydrolysed. Hydrogen sulfide is not produced. Simmons' citrate test is positive. Nitrate is not reduced. Acid is produced oxidatively from D-arabinose, D-glucose, D-galactose, D-mannose, fructose and maltose. Methanol, formate and formaldehyde are utilized as sole carbon sources. Ammonium sulfate, nitrate, aspartate and glutamate are utilized as sole nitrogen sources. The following compounds are utilized as sole carbon and energy sources (Biolog): D-fructose, acetic acid, α -, β - and γ -hydroxybutyric acid, α -ketoglutaric acid, L-lactic acid, D- and L-malic acid, methyl pyruvate, monomethyl succinate, propionic acid, pyruvic acid, succinamic acid, succinic acid, L-glutamic acid and glycerol. The following compounds are not utilized as sole carbon and energy sources (Biolog): Tweens 40 and 80, α -cyclodextrin, β -cyclodextrin, dextrin, glycogen, inulin, mannan, N-acetyl-D-glucosamine, N-acetyl-D-mannosamine, amygdalin, L-arabinose, D-arabitol, arbutin, cellobiose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic

acid, α -D-glucose, *myo*-inositol, α -D-lactose, lactulose, maltose, maltotriose, D-mannitol, D-mannose, D-melezitose, D-melibiose, methyl α -D-galactoside, methyl β -D-galactoside, 3-methyl glucose, methyl α -D-glucoside, methyl β -D-glucoside, methyl α -D-mannoside, palatinose, D-psicose, D-raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-tagatose, D-trehalose, turanose, xylitol, D-xylose, *p*-hydroxyphenylacetic acid, α -ketovaleric acid, lactamide, D-lactic acid methyl ester, N-acetyl-L-glutamic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, glycyl L-glutamic acid, L-pyroglutamic acid, L-serine, putrescine, 2,3-butanediol, adenosine, 2'-deoxyadenosine, inosine, thymidine, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate, uridine 5'-monophosphate, fructose 6-phosphate, glucose 1-phosphate, glucose 6-phosphate and DL- α -glycerol phosphate.

The type strain, AR27^T (= CECT 7069^T = CCM 7305^T), was isolated from drinking water. The DNA G + C content of the type strain is 63.6 mol% (T_m).

Acknowledgements

V. G. was supported by a fellowship from the Spanish Ministerio de Educación y Ciencia. We thank Y. Kato for his comments concerning the phylogenetic analysis. This work was supported by grants from the Quality of Life and Management of Living Resources Programme of the European Commission (QLK3-CT-2002-01972), Spanish Ministerio de Ciencia y Tecnología (BMC2003-01344) and from the Junta de Andalucía.

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