Methylobacterium iners sp. nov. and *Methylobacterium aerolatum* sp. nov., isolated from air samples in Korea

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Two bacterial strains isolated from air samples, 5317S-33^T and 5413S-11^T, were characterized by determining their phenotypic characteristics, cellular fatty acid profiles and phylogenetic positions based on 16S rRNA gene sequence analysis. 16S rRNA gene sequence analysis showed that these isolates belonged to the genus *Methylobacterium*. Strain 5317S-33^T was most closely related to *Methylobacterium adhaesivum* AR27^T (97.9% sequence similarity). Strain 5413S-11^T was most closely related to *Methylobacterium fujisawaense* DSM 5686^T (97.3% sequence similarity), *Methylobacterium oryzae* CBMB20^T (97.1% similarity) and *Methylobacterium radiotolerans* JCM 2831^T (97.0% similarity). Cells of both strains were strictly aerobic, Gram-negative, motile and rod-shaped. The major fatty acid was C_{18:1ω7c}. The G+C contents of the genomic DNA were 68.0 mol% for strain 5317S-33^T and 73.2 mol% for strain 5413S-11^T. According to DNA–DNA hybridization data, strain 5317S-33^T showed a level of DNA–DNA relatedness of 33% with *M. adhaesivum* DSM 17169^T, and strain 5413S-11^T showed low levels of DNA–DNA relatedness (<35%) with *M. fujisawaense* DSM 5686^T, *M. oryzae* CBMB20^T and *M. radiotolerans* DSM 1819^T. On the basis of this polyphasic analysis, it was concluded that strains 5317S-33^T and 5413S-11^T represent two novel species within the genus *Methylobacterium*, for which the names *Methylobacterium iners* sp. nov. (type strain 5317S-33^T = KACC 11765^T = DSM 19015^T) and *Methylobacterium aerolatum* sp. nov. (type strain 5413S-11^T = KACC 11766^T = DSM 19013^T) are proposed.

The genus *Methylobacterium* was originally proposed by Patt *et al.* (1976). This genus was characterized as containing strictly aerobic, Gram-negative and pink-pigmented facultatively methylotrophic bacteria (Green, 1992). Members of the genus *Methylobacterium* have been isolated mainly from soil and water (Corpe & Rheem, 1989; Lidstrom & Chistoserdova, 2002).

In the course of study of the bacterial population in air samples, two pink-coloured bacterial strains were isolated. Air samples were collected in the Suwon region, Republic

of Korea, using an MAS-100 air sampler (single-stage multiple-hole impactor; Merck). The sampler contained Petri dishes with R2A agar (BBL) amended with 0.02% cycloheximide (Sigma). After sampling, plates were incubated at 28 °C for 5 days and strains 5317S-33^T and 5413S-11^T were recovered. Routine cultivation was conducted at 28 °C with R2A media.

Cell morphology was examined by light microscopy (AXIO; Zeiss). The test for motility was performed on one-tenth-strength R2A broth 2216 (Difco) supplemented with 0.2% agar. Anaerobic growth was tested by incubating cultures on R2A plates in a GasPak jar (BBL) for 14 days, with aerobically incubated plates as controls. Growth at different temperatures (5–45 °C) and pH values (4, 5, 6, 7, 8, 9 and 10) was monitored in R2A broth. The requirement for NaCl was tested in R2A broth supplemented with 0, 2, 3, 5 and 7% (w/v) NaCl. Gram-staining,

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 5317S-33^T and 5413S-11^T are EF174497 and EF174498, respectively.

Cellular fatty acid compositions of strains 5317S-33^T and 5413S-11^T and related *Methylobacterium* species are detailed as supplementary material available with the online version of this paper.

catalase and oxidase activities and hydrolysis of aesculin, casein, CM-cellulose, DNA, hypoxanthine, tyrosine, Tween 80, starch and xanthine were determined as described by Smibert & Krieg (1994). For the various physiological tests, API 20NE and API ZYM test strips (bioMérieux) were used according to the manufacturer's instructions. Biolog GN2 plates were used to test activity against 95 carbon substrates. Both strains were strictly aerobic, Gram-negative, motile and rod-shaped. Strain 5317S-33^T grew on R2A (Difco) and R2A plus 0.5 % methanol, but did not grow on nutrient agar (NA; Difco), NA plus 0.5 % methanol, trypticase soy agar (Difco) or MacConkey agar (Difco). Strain 5413S-11^T grew on R2A (Difco), R2A plus 0.5 % methanol, NA and NA plus 0.5 % methanol, but did not grow on trypticase soy agar or MacConkey agar. Both strains could be differentiated clearly from their close relatives by several phenotypic characteristics such as nitrate reduction, urease, gelatin hydrolysis, substrate assimilation and enzyme activities (Table 1).

Whole-cell fatty acids were analysed according to the standard protocol of the MIDI/Hewlett-Packard Microbial

Identification system (Sasser, 1990). DNA G + C contents were determined according to the method of Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18 S; Supelco). Both strains had C_{18:1}ω7c as the major fatty acid, which was in agreement with the fatty acid profiles of other *Methylobacterium* species (Supplementary Table S1, available in IJSEM Online). The DNA G + C content of strain 5317S-33^T was 68.0 mol%; that of strain 5413S-11^T was 73.2 mol%.

The 16S rRNA gene was amplified by using PCR with primers fD1 and rP2 (Weisburg *et al.*, 1991) on colonies; the entire PCR fragment was sequenced directly (Hiraishi, 1992). The resultant 16S rRNA gene sequences were compared with available sequences from GenBank using the program BLAST (<http://www.ncbi.nlm.nih.gov/blast/>) to determine an approximate phylogenetic affiliation and gene sequences were aligned with those of closely related strains using the software CLUSTAL W (Thompson *et al.*, 1994). Phylogenetic trees were constructed by the neighbour-joining method with the program package MEGA3 (Kumar *et al.*, 2004). Bootstrap confidence values were obtained using 1000 resamplings. Phylogenetic trees were also constructed by using the maximum-parsimony method. DNA–DNA hybridization was carried out as described by Seldin & Dubnau (1985). Probe labelling was conducted using a non-radioactive DIG High Prime DNA Labelling and Detection Starter kit II (Roche Molecular Biochemicals). Hybridized DNA was visualized using a DIG luminescent detection kit (Roche Molecular Biochemicals). The 16S rRNA gene sequence similarities between strain 5317S-33^T and the type strains of all recognized *Methylobacterium* species were in the range 93.6–97.9%; its closest relative was *Methylobacterium adhaesivum* AR27^T (97.9% sequence similarity). Strain 5413S-11^T showed sequence similarity levels of 91.6–97.3% with all type strains of the genus *Methylobacterium*. It revealed >97% sequence similarity with *Methylobacterium fujisawaense* DSM 5686^T (97.3%), *Methylobacterium oryzae* CBMB20^T (97.1%) and *Methylobacterium radiotolerans* JCM 2831^T (97.0%). According to the neighbour-joining tree (Fig. 1), strain 5317S-33^T formed a compact cluster with *M. adhaesivum* AR27^T (99% bootstrap support). Strain 5413S-11^T clustered with *M. fujisawaense*, *M. oryzae*, *M. radiotolerans* and *Methylobacterium mesophilicum*. The topologies of phylogenetic trees built using the maximum-parsimony algorithm also supported the notion that these isolates belong to the genus *Methylobacterium* (data not shown). Strain 5317S-33^T showed a level of DNA–DNA relatedness of 33% with *M. adhaesivum* DSM 17169^T. Strain 5413S-11^T showed low levels of DNA–DNA relatedness with *M. fujisawaense* DSM 5686^T (34%), *M. oryzae* CBMB20^T (28%) and *M. radiotolerans* DSM 1819^T (35%). On the basis of this polyphasic taxonomic analysis, the names *Methylobacterium iners* sp. nov. and *Methylobacterium aerolatum* sp. nov. are proposed to accommodate these two novel strains.

Table 1. Differential properties of strains 5317S-33^T and 5413S-11^T and closely related *Methylobacterium* species

Strains: 1, 5317S-33^T; 2, 5413S-11^T; 3, *M. adhaesivum* DSM 17169^T; 4, *M. fujisawaense* DSM 5686^T; 5, *M. mesophilicum* DSM 1708^T; 6, *M. oryzae* KACC 11585^T; 7, *M. radiotolerans* DSM 1819^T. According to the API 20NE test strip, all strains are negative for indole production, glucose fermentation, arginine dihydrolase, aesculin hydrolysis and β-galactosidase. None of the strains assimilate D-mannose, D-mannitol, N-acetylglucosamine, maltose, capric acid or phenylacetic acid. According to the API ZYM test strip, all strains are positive for esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; (+), weakly positive; –, negative.

Characteristic	1	2	3	4	5	6	7
Nitrate reduction	–	–	–	–	–	+	+
Urease	–	+	+	–	–	–	–
Gelatin hydrolysis	+	–	–	–	–	–	–
Assimilation of:							
D-Glucose	–	(+)	(+)	–	–	–	+
L-Arabinose	–	(+)	(+)	+	+	+	+
Potassium gluconate	–	(+)	–	+	+	+	+
Adipic acid	–	–	–	+	+	+	+
Malic acid	–	+	–	+	+	+	+
Trisodium citrate	–	+	+	–	+	–	–
Activity of:							
Alkaline phosphatase	+	+	+	–	–	–	–
Valine arylamidase	–	–	–	–	–	+	–
Trypsin	+	–	–	+	+	+	+
Acid phosphatase	–	+	–	+	+	+	+

Fig. 1. Phylogenetic tree showing the relationship between strains 5317S-33^T and 5413S-11^T and related species. The tree was constructed by using the neighbour-joining method based on 16S rRNA gene sequences. Numbers at nodes indicate percentage bootstrap values from 1000 replicates. Bootstrap values greater than 50 % are shown. Bar, 0.01 substitutions per nucleotide position.

Description of *Methylobacterium iners* sp. nov.

Methylobacterium iners (i'ners. L. neut. adj. *iners* inactive, lazy).

Cells are strictly aerobic, Gram-negative, motile and rod-shaped ($1.0 \times 1.5\text{--}2.5\ \mu\text{m}$). Colonies are pink-coloured, circular and convex with entire margins. Produces oxidase and catalase. Growth is observed at $10\text{--}30\ ^\circ\text{C}$ (optimum $25\text{--}28\ ^\circ\text{C}$) and pH $6.0\text{--}8.0$ (optimum 7.0). Cannot grow in the presence of NaCl concentrations of 2 % or more. Hydrolyses starch, but does not hydrolyse casein, CM-cellulose, DNA, hypoxanthine, Tween 80, tyrosine or xanthine. The following substrates produce positive results in Biolog GN2 plates: pyruvic acid methyl ester, acetic acid, formic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, α -ketobutyric acid, DL-lactic acid, propionic acid, succinamic acid, L-asparagine, L-aspartic acid and L-glutamic acid. The major fatty acid is $\text{C}_{18:1\omega7\text{c}}$.

The type strain is 5317S-33^T (=KACC 11765^T =DSM 19015^T), isolated from an air sample in Suwon city, Republic of Korea. The DNA G+C content of the type strain is 68.0 mol%.

Methylobacterium aerolatum sp. nov.

Methylobacterium aerolatum (ae.ro.la'tum. Gr. n. *aer* air; L. part. adj. *latus* carried; N.L. neut. part. adj. *aerolatum* airborne).

Cells are strictly aerobic, Gram-negative, motile and rod-shaped ($1.0 \times 1.5\text{--}3.0\ \mu\text{m}$). Colonies are pink-coloured, circular and convex with entire margins. Produces oxidase and catalase. Growth is observed at $10\text{--}35\ ^\circ\text{C}$ (optimum $28\ ^\circ\text{C}$) and pH $5.0\text{--}9.0$ (optimum 7.0). Cannot grow in the presence of NaCl concentrations of 2 % or more.

Hydrolyses starch, hypoxanthine and xanthine, but does not hydrolyse casein, CM-cellulose, DNA, Tween 80 or tyrosine. The following substrates produce positive results in Biolog GN2 plates: pyruvic acid methyl ester, succinic acid monomethyl ester, formic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketoglutaric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid and glycerol. The major fatty acid is $\text{C}_{18:1\omega7\text{c}}$.

The type strain is 5413S-11^T (=KACC 11766^T =DSM 19013^T), isolated from an air sample in Suwon city, Republic of Korea. The DNA G+C content of the type strain is 73.2 mol%.

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