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NOTE

Microbacterium suwonense sp. nov., Isolated from Cow Dung[§]

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An actinomycete strain, designated M1T8B9^T, was isolated from cow dung in Suwon, Republic of Korea. The isolate was a Gram-positive, nonmotile, and non-spore-forming bacterium. Phylogenetic evaluation based on 16S rRNA gene sequence similarity showed that this isolate belongs to the genus *Microbacterium*, with its closest neighbors being *Microbacterium soli* DCY17^T (98.2%) and *Microbacterium esteraromaticum* DSM 8609^T (98.0%). The polar lipid pattern consisted of diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid. Strain M1T8B9^T contained the major fatty acids $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso, and the cell-wall peptidoglycan was of type B2β. According to DNA-DNA hybridization studies, strain M1T8B9^T showed 42% and 26% relatedness with *M. soli* DCY17^T and *M. esteraromaticum* DSM 8609^T, respectively. On the basis of the data presented, strain M1T8B9^T is considered to represent a novel species of the genus *Microbacterium*, for which the name *Microbacterium suwonense* sp. nov. is proposed. The type strain is M1T8B9^T (=KACC 14058^T =NBRC 106310^T).

***Keywords*:** *Microbacterium suwonense* sp. nov., taxonomy, 16S rRNA gene, phylogeny, new species

The genus *Microbacterium* was first described by Orla-Jensen (1919), and taxonomic reevaluations of this genus were conducted by Collins *et al.* (1983) and Takeuchi and Hatano (1998a). The latter incorporated the genus *Aureobacterium* within the genus *Microbacterium*. Members of the genus *Microbacterium* are characterized by the presence of L-lysine or D-ornithine in the peptidoglycan (Schumann *et al.*, 2009). The major isoprenoid quinones are menaquinones with 10-14 (mainly 11-13) unsaturated isoprenoid units, and the DNA G+C content is 64-75 mol%. These organisms have been isolated from various environmental habitats, including air, plants, soil, water, milk products, and also from humans (Evtushenko and Takeuchi, 2006; Wu *et al*., 2008).

Bacterial strains were isolated from cow dung by serial dilution plating on tryptic soy agar (TSA), nutrient agar (NA), Luria-Bertani agar (LB), and R2A agar (all from Difco, USA). Plates were incubated for 4 days at 30°C. Fifty-four bacterial strains were isolated (data not shown), of which strain M1T8B9^T appeared on LB agar medium.

The cell morphology of strain M1T8B9^T was examined by light microscopy (AXIO; Zeiss), and transmission electron (LEO model 912AB) microscopy with cells grown for 3 days on R2A agar at 30°C. For transmission electron microscopy, cells were negatively stained with 0.5% (w/v) uranyl acetate. Optimum conditions for growth were determined by culturing at a variety of temperatures, pH values, and NaCl concentrations for up to 7 days. Growth was monitored on R2A at 4, 10, 15, 20, 25, 30, 37, 40, and 45°C. Optimum pH was determined in R2A adjusted to pH 5.0-10.0 in increments of 1.0 units. NaCl tolerance was tested by culturing on R2A containing NaCl at various concentrations (0, 1, 2, 3, 5, and 7%, w/v). Gram staining, catalase, oxidase, and casein, CM-cellulose, DNA, starch, tyrosine, and Tween 80 hydrolysis assays were conducted as described by Smibert and Krieg (1994). Motility testing was performed on one-tenth strength R2A broth supplemented with 0.2% agar. Anaerobic growth was investigated using incubation in the BBL GasPak Anaerobic System (Difco) for 10 days at 30°C on R2A agar. Enzyme activities, utilization of carbon sources and acid production from substrates were tested with commercial API ZYM, API 20NE, API ID 32GN, and API 50CH kits, according to the manufacturer's protocols. Cells of strain M1T8B9^T were Gram-positive, aerobic, nonmotile, and coccus or short rod-shaped. Colonies were white-colored with a round, convex shape after 3 days of growth at 30°C, and became irregular after 5 days of growth at 30°C on R2A agar. Growth occurred on R2A, NA, TSA, and MacConkey agar.

Isolation of chromosomal DNA, PCR amplification, and direct sequencing of the purified product were carried out as described previously (Weon *et al.*, 2006). The resultant 16S rRNA gene sequence (1,425 bp) was aligned with the corresponding sequences of some members of the genus *Microbacterium*

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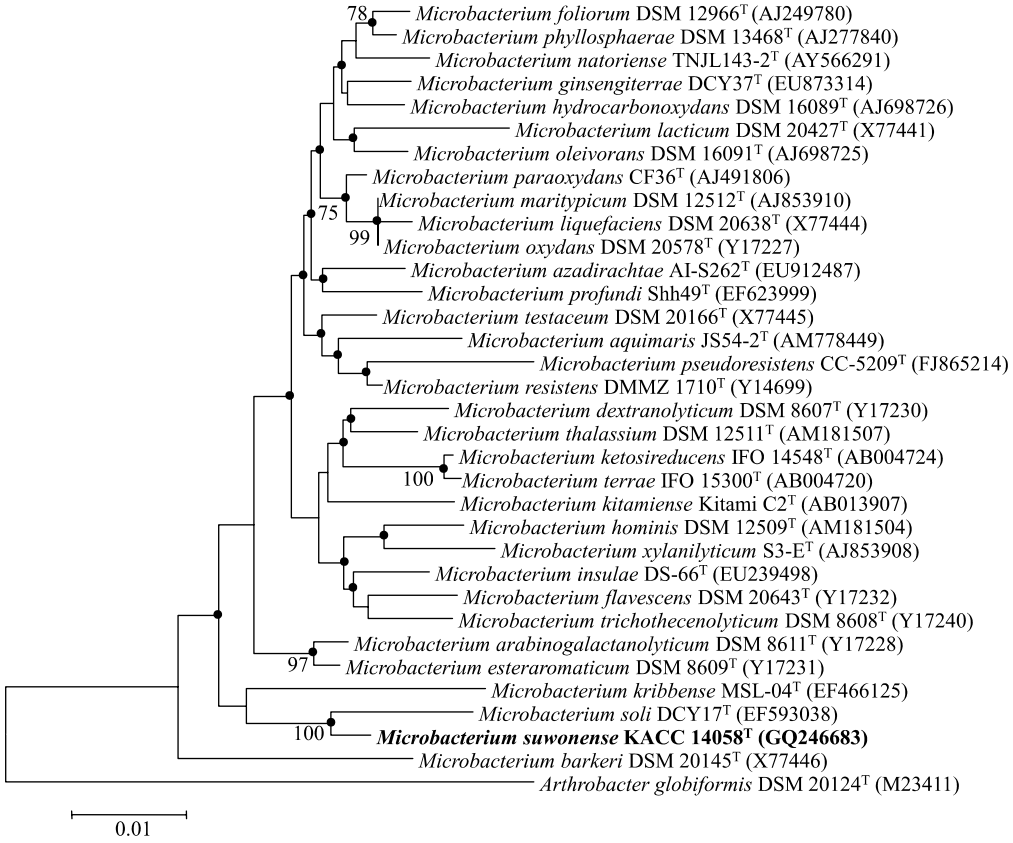

Fig. 1. Phylogenetic tree reconstructed by the neighbour-joining method showing the clustering of strain M1T8B9^T with other species of the genus *Microbacterium*. *Arthrobacter globiformis* DSM 20124^T was used as an outgroup. GenBank accession nos. are given in parentheses. Numbers represent confidence levels (percentages higher than 70% are shown) from 1,000 replicate bootstrap resamplings. Closed circles indicate those branches also found in the tree reconstructed by the maximum-parsimony method. Bar, 0.01 substitutions per nucleotide position.

retrieved from the GenBank database using the CLUSTAL W program (Thompson *et al.*, 1994). A phylogenetic tree was produced using the MEGA version 3.1 software package (Kumar *et al.*, 2004). Distances (using distance options according to Kimura's two-parameter model) and clustering using the neighbor-joining and maximum-parsimony methods were determined using bootstrap values based on 1,000 replicates. In the neighbor-joining phylogenetic tree based on 16S rRNA gene sequences, strain M1T8B9^T fell within the clade comprising species of the genus *Microbacterium* and formed a coherent cluster with *Microbacterium soli* DCY17^T on the basis of 100% bootstrap values (Fig. 1). The same cluster was found in trees reconstructed using maximum-parsimony algorithms (Fig. 1). Strain M1T8B9^T exhibited the highest 16S rRNA gene sequence similarities (98.2% and 98.0%, respectively) with *M. soli* DCY17^T and *Microbacterium esteraromaticum* DSM 8609^T, and showed less than 98% sequence similarities with type strains of other species within the genus *Microbacterium*.

Analysis of cell-wall and fatty acids was conducted using cells grown for 48 h on TSA at 30°C. Chemotaxonomic analyses for the following substrates were performed as described: peptidoglycan (Schleifer and Kandler, 1972), polar lipids (Minnikin *et al.*, 1984), and fatty acids (according to the standard protocol of the Sherlock Microbial Identification System; Sasser, 1990). DNA-DNA hybridization was carried out as described by Seldin and Dubnau (1985). Probe labeling was conducted using the nonradioactive DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Molecular Biochemicals, USA). Reassociation was conducted at 65°C. The hybridized DNA was visualized using the DIG luminescent detection kit (Roche Molecular Biochemicals). DNA-DNA relatedness was quantified with a densitometer (Bio-Rad, USA). DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989). Hydrolysates of the purified peptidoglycan of strain M1T8B9^T contained the amino acids ornithine (Orn), alanine (Ala), glycine (Gly), homoserine (Hsr), and 3-hydroxy glutamic acid (Hyg) at an approximate molar ratio of 1.1:1.2:1.6:1.0:0.7, supporting the view that strain M1T8B9^T contains type B2β peptidoglycan (Schleifer and Kandler, 1972). The major polar lipids were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and one unknown glycolipid (GL) (Supplementary data Fig. 1). M1T8B9^T contained the fatty acids (>10% of total fatty acids) $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso (Table 2). Although the threshold value for 16S rRNA gene similarity of two different species is proposed to be <98.7% (Stackebrandt and Ebers, 2006), DNA-DNA hybridizations were performed using *M. soli* DCY17^T and *M. esteraromaticum* DSM 8609^T as the two closest relatives to strain M1T8B9^T.

Table 1. Differential phenotypic characteristics of M1T8B9^T and closely related *Microbacterium* species
Strains: 1, strain M1T8B9^T; 2, *Microbacterium soli* DCY 17^T (Srinivasan *et al*., 2010); 3, *Microbacterium esteraromaticum* DSM 8609^T (Yokota *et al.*, 1993; Zlamala *et al.*, 2002); 4, *Microbacterium arabinogalactanolyticum* DSM 8611^T (Yokota *et al.*, 1993; Zlamala *et al.*, 2002); 5, *Microbacterium trichothecenolyticum* DSM 8608^T (Yokota *et al.*, 1993; Yoon *et al*., 2009); 6, *Microbacterium xylanilyticum* KCTC 19079^T (Kim *et al.*, 2005; Yoon *et al*., 2009); 7, *Microbacterium hominis* DSM 12509^T (Takeuchi and Hatano, 1998b; Kim *et al.*, 2005; Yoon *et al*., 2009). All strains are catalase-positive. All strains have ornithine as the peptidoglycan diamino acid and have the B2β type of peptidoglycan bridge. All strains are positive for assimilation of D-mannitol, *N*-acetylglucosamine, D-maltose and potassium gluconate, and activity of α-glucosidase. All strains produce acid from sucrose, but do not from inulin. +, positive; -, negative; w, weakly positive; NA, no data available; W, white; PY, pale yellow; Y, yellow; YW, yellowish white.

Characteristics	1	2	3	4	5	6	7
Colony colour	W	PY	Y	W	Y	Y	YW
Oxidase	+	-	+	+	-	-	-
Motility	-	-	+	-	-	-	-
Growth at 37°C	+	+	NA	-	-	+	+
Nitrate reduction	+	-	-	+	+	+	-
Arginine dihydrolase	-	-	-	+	-	-	-
Urease	-	-	-	+	+	-	-
Gelatin hydrolysis	+	-	-	+	+	-	-
Assimilation of:							
Trisodium citrate	-	-	-	w	+	-	+
Phenylacetic acid	-	+	-	+	-	+	-
D-Ribose	+	-	NA	NA	-	+	-
Inositol	+	-	NA	NA	+	-	-
Lactic acid	-	-	+	+	NA	+	
L-Alanine	-	-	+	+	NA	-	
Salicin	-	+	NA	NA	+	+	+
Propionic acid	-	-	+	+	NA	-	
Enzyme activity							
Alkaline phosphatase	+	w	w	-	-	-	-
Esterase lipase (C8)	-	w	w	w	+	-	+
Valine arylamidase	-	+	+	w	-	-	-
Cystine arylamidase	-	+	+	w	-	-	-
Trypsin	-	+	w	-	-	-	-
α-Galactosidase	-	+	-	-	-	+	+
β-Glucosidase	-	+	w	+	+	-	+
N-Acetyl-β-glucosaminidase	-	-	-	+	+	-	+
α-Mannosidase	+	+	+	+	-	+	-
α-Fucosidase	-	-	+	-	-	-	-
Acid production from:							
Glycerol	-	-	+	+	+	+	+
D-Arabinose	-	+	w	-	-	+	-
L-Arabinose	+	+	w	-	+	+	+
D-Ribose	-	w	-	+	-	+	-
D-Xylose	+	+	-	+	+	+	+
Methyl-β-D-xylopyranoside	-	+	-	+	+	-	-
D-Mannose	-	w	w	+	+	+	+
L-Rhamnose	+	-	-	+	+	-	+
D-Mannitol	+	+	-	+	+	+	+
Methyl-α-D-glucopyranoside	-	+	w	-	-	+	+
Amygdalin	-	-	w	+	+	+	+
Arbutin	-	-	-	+	+	-	+
Salicin	-	-	w	+	+	+	+
D-Lactose	-	-	+	w	+	+	+
D-Melibiose	-	-	-	w	+	+	+
Amidon	+	-	+	-	+	+	+
Glycogen	+	-	+	+	+	+	+
Potassium gluconate	-	+	-	+	+	+	+
DNA G+C content (mol%)	69.6	70.2	68.8	69.3	69.0	69.7	71.2

Table 2. Cellular fatty acid profiles of strain M1T8B9^T and the type strains of related *Microbacterium* species
Strains: 1, strain M1T8B9^T; 2, *Microbacterium soli* DCY 17^T (Srinivasan *et al*., 2010); 3, *Microbacterium esteraromaticum* DSM 8609^T (Srinivasan *et al*., 2010); 4, *Microbacterium arabinogalactanolyticum* DSM 8611^T (Srinivasan *et al*., 2010); 5, *Microbacterium xylanilyticum* DSM 16914^T (Srinivasan *et al*., 2010); 6, *Microbacterium hominis* DSM 12509^T (Takeuchi and Hatano, 1998b). -, <1.0% or not detected.

Fatty acids	1	2	3	4	5	6
$C_{14:0}$ iso	1.4	2.1	2.3	2.5	2.0	-
$C_{15:0}$ anteiso	34.4	32.5	56.6	35.5	42.6	40.9
$C_{15:0}$ iso	13.3	27.5	14.8	28.9	6.1	5.7
$C_{16:0}$	3.1	1.0	2.0	1.9	2.9	6.7
$C_{16:0}$ iso	22.8	17.0	12.2	15.7	32.5	21.0
$C_{17:0}$ anteiso	19.4	13.2	9.7	10.0	12.0	23.5
$C_{17:0}$ iso	4.5	6.1	1.6	4.4	1.4	2.3

The DNA-DNA relatedness values of strains M1T8B9^T and *M. soli* DCY17^T and *M. esteraromaticum* DSM 8609^T were 42% and 26%, respectively. The DNA G+C content of strain M1T8B9^T was 69.6 mol%.

Strain M1T8B9^T should be recognized as a member of *Microbacterium* on the basis of the phylogenetic, phenotypic, and chemotaxonomic data mentioned above. However, strain M1T8B9^T can be clearly differentiated from closely related species of the genus *Microbacterium* on the basis of phenotypic properties and DNA-DNA relatedness. M1T8B9^T showed phenotypic properties (colony color, oxidase, nitrate reduction, gelatin hydrolysis, assimilations of substrates, acid productions from substrates, and enzymatic activities) different from those of its closest relative, *M. soli* DCY17^T (Table 1). Additionally, strain M1T8B9^T can be clearly differentiated from closely related species by comparing the characteristics in Table 1. Strain M1T8B9^T showed lower (≤42%) DNA relatedness with its closest relatives in the genus *Microbacterium* than the 70% threshold value for species demarcation (Wayne *et al*., 1987).

Based on a phylogenetic analysis and phenotypic characteristics, strain M1T8B9^T is described as a novel species in the genus *Microbacterium*, for which the name *Microbacterium suwonense* sp. nov. (type strain M1T8B9^T =KACC 14058^T =NBRC 106310^T) is proposed.

Description of *Microbacterium suwonense* sp. nov.

Microbacterium suwonense (su.won.en'se. N.L. neut. adj. *suwonense* referring to the Suwon region, Republic of Korea, where the type strain was first isolated).

Cells are Gram-positive, aerobic, nonmotile, and coccus or short rod-shaped (about 0.5-0.7 μm wide and 0.7-2.5 μm long) after 3 days of growth on R2A at 30°C. Colonies are white-colored, and round and convex shaped after 3 days of growth at 30°C, and become irregular after 5 days of growth at 30°C on R2A. Growth occurs on R2A, NA, TSA, and MacConkey agar. The strain grows at 10-40°C and pH 6.0-8.0; optimum growth occurs at 30°C and pH 7.0, and does not require NaCl for growth, but can tolerate up to 3% (w/v) NaCl. It hydrolyzes starch, but not casein, CM-cellulose, DNA, tyrosine, or Tween 80, and it is catalase- and oxidase-positive. The strain is positive for nitrate reduction, aesculin hydrolysis, gelatin hydrolysis, and β-galactosidase (PNG), but negative for idole production, glucose fermentation, arginine hydrolase, and urease (API 20NE test strip). It assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, D-ribose, inositol, D-saccharose, glycogen, and L-proline, but not other substrates embedded on test strips (API 20NE and API ID 32GN). It is positive for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, and α-mannosidase activities, but not for esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, or α-fucosidase (API ZYM). Acid is produced from L-arabinose, D-xylose, L-rhamnose, D-mannitol, aesculin ferric citrate, D-cellobiose, D-maltose, D-saccharose, D-trehalose, amidon, glycogen, and D-turanose, and weakly from L-fucose but not from other substrates on the test strip (API 50CH). The peptidoglycan is of type B2β with an interpeptide bridge Hyg → Gly → D-Orn. The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, and one unknown glycolipid. Major fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$ iso, $C_{17:0}$ anteiso, and $C_{15:0}$ iso. The DNA G+C content of strain M1T8B9^T was 69.6 mol%.

The type strain, M1T8B9^T (=KACC 14058^T =NBRC 106310^T), was isolated from cow dung in Suwon, Republic of Korea.

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