Microbacterium soli sp. nov., an α -glucosidase-producing bacterium isolated from soil of a ginseng field

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Five Gram-type-positive, aerobic, rod-shaped, non-motile strains of *Microbacterium* (DCY 17^T, Ms1, Ms2, Ms3 and Ms4) were isolated from soil from a ginseng field in Daejeon, South Korea. On the basis of 16S rRNA gene sequence similarity, these strains were shown to be related to *Microbacterium esteraromaticum* DSM 8609^T (96.1%), *M. xylanilyticum* DSM 16914^T (96.0%), *M. aquimaris* JS54-2^T (95.6%), *M. insulae* DS-66^T (95.5%), *M. ketosireducens* IFO 14548^T (95.5%) and *M. arabinogalactanolyticum* DSM 8611^T (95.4%). Chemotaxonomic data revealed that the type strain, DCY 17^T, possesses menaquinones MK-12, MK-11 and MK-13 and the predominant fatty acids C_{15:0} anteiso (32.5%), C_{15:0} iso (27.5%), C_{16:0} iso (17.0%), C_{17:0} anteiso (13.2%), C_{17:0} iso (6.1%) and C_{14:0} iso (2.1%). The DNA G + C content of strain DCY 17^T is 70.2 mol% and those of strains Ms1 to Ms4 are in the range 68.9–73.5 mol%. The physiological and biochemical tests suggested that these strains represent a novel species. Based on these data, DCY 17^T (=KCTC 19237^T =LMG 24010^T) is classified as the type strain of a novel *Microbacterium* species, for which the name *Microbacterium soli* sp. nov. is proposed.

The genus *Microbacterium* (phylum *Actinobacteria*, class *Actinobacteria*, order *Actinomycetales*, family *Microbacteriaceae*) was described by Orla-Jensen (1919) and its description was emended by Collins *et al.* (1983). More recently, the description of the genus was emended again to unite the genera *Microbacterium* and *Aureobacterium* (Takeuchi & Hatano, 1998a). At the time of writing, the genus *Microbacterium* consisted of 62 species with validly published names. In this paper, we have characterized new isolates belonging to the genus *Microbacterium* using a polyphasic taxonomic approach. The phenotypic and genotypic characterization of the novel strains is described in this report.

In a series of studies, we have attempted to isolate micro-organisms from soil in order to investigate the community structure based on a culture-dependent method. In this study, five strains were isolated from soil in a ginseng field

in Daejeon city, South Korea, and characterized by a polyphasic approach, including 16S rRNA gene sequence analysis, genotypic relatedness and chemotaxonomic and phenotypic properties. The results obtained in this study indicated that strains DCY 17^T and Ms1 to Ms4 can be assigned to a novel species of the genus *Microbacterium*. Strains were isolated from surface soil of an agricultural field where ginseng was planted. One gram of the soil was immersed in 50 ml saline solution, vortexed and serially diluted and 100 μ l aliquots were inoculated onto tenfold-diluted R2A agar (Difco). Purified colonies were tentatively identified by using partial 16S rRNA gene sequences.

Cell morphology and motility were observed with a Nikon light microscope ($\times 1000$ magnification) using 5-day-old cells grown on R2A agar at 30 °C. The Gram reaction was conducted according to the non-staining method, as described by Buck (1982). Oxidase activity was evaluated via the oxidation of 1% *p*-aminodimethylaniline oxalate. Catalase activity was determined by the measurement of bubble production after the addition of 3% (v/v) hydrogen peroxide solution (Cappuccino & Sherman, 2002). Growth at 4, 15, 18, 25, 30, 37 and 42 °C was assessed on R2A agar and growth at pH 5.0–10.0 (at intervals of 1 pH unit) was determined in R2A broth. Anaerobic growth was investigated in serum bottles by adding thioglycolate (1 g l⁻¹) to

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strain DCY 17^T and strains Ms1 to Ms4 are respectively EF593038–EF593042.

Fatty acid profiles of strain DCY 17^T and related type strains and detailed DNA–DNA hybridization results are available as supplementary material with the online version of this paper.

Table 1. Differential phenotypic characteristics between strains of *Microbacterium soli* sp. nov. and related *Microbacterium* species

Strains: 1, *M. soli* sp. nov. DCY 17^T; 2–5, *M. soli* Ms1–Ms4; 6, *M. aquimaris* JS54-2^T (data from Kim *et al.*, 2008); 7, *M. arabinogalactanolyticum* DSM 8611^T (Zlamala *et al.*, 2002; Yokota *et al.*, 1993); 8, *M. esteraromaticum* DSM 8609^T (Zlamala *et al.*, 2002; Yokota *et al.*, 1993); 9, *M. halophilum* IFO 16062^T (Takeuchi & Hatano, 1998b); 10, *M. xylanilyticum* DSM 16914^T (Kim *et al.*, 2005a). All strains are Gram-positive, catalase-positive and positive for assimilation of maltose and mannitol. All strains have ornithine as the peptidoglycan diamino acid and have the B2 β type of peptidoglycan bridge (not determined for strains Ms1–Ms4). +, Positive; –, negative; w, weakly positive; ND, not determined; NA, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10
Motility	–	–	–	–	–	–	–	+	–	–
Oxidase	–	–	–	–	–	+	+	+	NA	+
Production of acid from glucose	w	–	+	w	–	+	+	w	+	+
Cell-wall sugar(s)*	Gal	ND	ND	ND	ND	Glc, Gal	Gal	Glc, Gal	Man, Gal, Glc	Glc, Gal
Production of H ₂ S	–	–	–	–	–	–	+	+	–	–
Enzyme activity										
N-Acetyl- β -glucosaminidase	–	–	–	w	w	+	+	–	+	–
Acid phosphatase	+	+	+	+	+	–	+	+	+	+
Alkaline phosphatase	w	w	w	w	w	–	–	w	–	+
Arginine dihydrolase	–	–	–	–	–	–	+	–	–	w
α -Chymotrypsin	w	w	w	w	w	–	–	–	w	w
Cystine arylamidase	+	+	+	+	+	+	w	+	+	+
Esterase (C8)	w	w	w	w	w	w	w	w	+	w
α -Fucosidase	–	–	–	–	–	+	–	+	–	+
α -Galactosidase	+	+	+	+	+	w	–	–	–	+
α -Glucosidase (starch hydrolysis)	+	+	+	+	+	+	+	+	–	+
β -Galactosidase (PNPG)	+	+	+	+	+	–	+	+	–	+
β -Glucosidase (aesculin hydrolysis)	+	+	+	+	+	+	+	w	w	+
α -Mannosidase	+	+	+	+	+	–	+	+	–	+
Naphthol-AS-BI-phosphohydrolase	w	w	w	w	w	+	+	w	w	+
Protease (gelatin hydrolysis)	–	–	–	–	–	–	+	–	+	w
Trypsin	+	+	w	–	w	–	–	w	w	+
Valine arylamidase	+	+	+	w	+	w	w	+	w	+
Acid production from:										
5-Ketogluconate	–	w	w	–	–	+	w	w	–	–
Gluconate	+	–	+	–	–	+	+	–	w	–
D-Arabinose	+	w	–	+	–	–	–	w	–	w
L-Arabinose	+	w	+	+	–	–	–	w	+	w
D-Fructose	w	–	+	w	–	+	w	–	+	w
D-Fucose	–	–	–	–	–	–	w	–	–	–
D-Galactose	w	–	–	–	–	+	w	–	+	w
Gentiobiose	w	–	w	–	–	–	–	–	w	–
Lactose	–	+	–	–	–	–	w	+	w	+
Maltose	w	+	w	–	w	+	+	+	+	+
D-Mannose	w	–	+	–	–	+	+	w	+	w
Melezitose	w	–	w	–	–	–	–	–	–	+
Melibiose	–	w	–	–	w	–	w	–	–	w
L-Rhamnose	–	–	–	–	–	–	+	–	+	w
Ribose	w	+	w	–	–	+	+	–	–	–
Sucrose	+	w	+	–	+	NA	+	+	+	–
D-Tagatose	–	–	–	–	–	–	w	–	w	–
Trehalose	+	+	+	+	+	–	w	+	–	+
Turanose	+	+	+	–	+	+	w	+	w	+
D-Xylose	+	+	w	+	–	+	+	–	+	+
Methyl α -D-glucoside	+	w	–	w	+	–	–	w	–	+
Methyl β -D-xyloside	+	–	–	–	–	+	+	–	–	–
Glycerol	–	–	–	–	–	+	+	+	–	–
Mannitol	+	+	+	w	–	+	+	–	+	w

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9	10
Sorbitol	—	—	—	—	—	—	w	—	w	—
Xylitol	—	—	—	—	—	—	—	—	+	—
N-Acetylglucosamine	—	—	—	w	w	—	w	w	—	—
Amygdalin	—	—	—	—	w	—	+	w	+	w
Arbutin	—	—	—	—	w	+	+	—	+	—
Inulin	—	—	—	—	—	—	—	—	w	—
Salicin	—	—	—	—	w	+	+	w	+	+
Glycogen	—	—	—	—	—	—	+	+	—	+
Starch	—	—	—	—	—	—	—	+	+	+
Assimilation of:										
Acetate	—	—	—	—	—	—	+	+	—	—
Citrate	—	—	—	—	—	—	w	—	—	—
Gluconate	+	+	+	+	+	—	+	+	+	+
DL-Lactate	—	—	—	—	—	—	+	+	—	+
Phenylacetate	+	+	+	+	+	—	+	—	—	w
Propionate	—	—	—	—	—	—	+	+	—	—
L-Alanine	—	—	—	—	—	—	+	+	w	—
N-Acetyl-D-glucosamine	+	+	+	+	+	—	+	+	—	+
Menaquinones†	12, 11, 13	12, 11, 13	12, 11, 13	12, 11, 13	12, 11, 13	11, 12, 10	12, 13	12, 13	11, 12, 13	12, 11, 13
DNA G + C content (mol%)	70.2	68.9	71.5	70.3	73.5	69.3	69.3	68.8	67.2	69.7

*Gal, Galactose; Glc, glucose; Man, mannose.

†In decreasing order of abundance.

R2A broth and replacing the upper, air layer with nitrogen gas. The API 20NE, API ZYM and API ID32GN microtest systems were used according to the manufacturer's instructions (bioMérieux). α -Glucosidase production was examined based on the hydrolysis of starch (1%) on R2A agar at 30 °C for 3 days.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v) and purified by TLC and subsequently analysed by HPLC, as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). In order to perform cellular fatty acid methyl ester analysis, the strain was grown on tryptic soy agar (TSA; Difco) for 48 h at 30 °C and then two loopfuls of the well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified with the Sherlock Microbial Identification System (MIDI, Inc.) (Sasser, 1990). Polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977). The amino-acid composition of the cell-wall peptidoglycan was determined by using TLC after hydrolysis with 6 M HCl at 100 °C for 18 h as described by Komagata & Suzuki (1987). Cell-wall sugars were analysed as described by Stanek & Roberts (1974).

For determination of the DNA G+C content, genomic DNA was extracted and purified with the Qiagen Genomic-tip system 100/G and then degraded enzymically into nucleosides. The nucleosides were analysed using HPLC as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA–DNA hybridization was performed

fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells. The hybridization was conducted in five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the means of the remaining three values are quoted as DNA–DNA relatedness values.

The 16S rRNA gene was amplified from chromosomal DNA of strain DCY 17^T using the universal bacterial primers 9F and 1512R (Weisburg *et al.*, 1991) and purified PCR products were sequenced by Genotec (Daejeon, Korea) (Kim *et al.*, 2005b). The full sequence of the 16S rRNA gene was compiled with SeqMan software, and the 16S rRNA gene sequence of the test strain was edited using the BioEdit program (Hall, 1999). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). The phylogenetic tree was constructed via the neighbour-joining method (Saitou & Nei, 1987) in the MEGA 3 program (Kumar *et al.*, 2004). Bootstrap analysis with 1000 replicates was also conducted, in order to obtain confidence levels for the branches (Felsenstein, 1985). All species of *Microbacterium* as well as representatives of related genera were included in the phylogenetic tree.

Strain DCY 17^T was an aerobic, Gram-type-positive, rod-shaped (0.5–0.9 × 0.2–0.5 µm), non-motile bacterium.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strains of *Microbacterium soli* sp. nov. and other species of *Microbacterium*. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate common nodes recovered with the maximum-parsimony algorithm.

Colonies on R2A agar (Difco) were pale yellow, circular and slightly convex after 3 days of cultivation at 30 °C. Strain DCY 17^T grew at 25–42 °C. The physiological characteristics of strain DCY 17^T are summarized in the species description and a comparison of selected characteristics of the five novel strains with related type strains is shown in Table 1.

The cellular fatty acid profiles of strain DCY 17^T and related *Microbacterium* type strains are shown in Supplementary Table S1, available in IJSEM Online. The cellular fatty acids

of strain DCY 17^T were C_{13:0} iso (0.1 %), C_{14:0} (0.2 %), C_{14:0} iso (2.1 %), C_{15:0} anteiso (32.5 %), C_{15:0} iso (27.5 %), C_{16:0} (1.0 %), C_{16:0} iso (17.0 %), C_{17:0} anteiso (13.2 %), C_{17:0} iso (6.1 %), C_{15:1} anteiso (0.1 %) and C_{16:0} iso 3-OH (0.1 %). The major menaquinones of strain DCY 17^T were MK-12, MK-11 and MK-13. Strain DCY 17^T exhibited major amounts of diphosphatidylglycerol and phosphatidylethanolamine, moderate amounts of phosphatidylglycerol and minor amounts of unknown amino lipids. The peptidoglycan contained the amino acids ornithine, glycine, alanine and aspartic acid. Analytical data suggested the presence of

peptidoglycan type B2 β in strain DCY 17^T. Galactose and traces of an unknown sugar were detected as cell-wall sugars.

The 16S rRNA gene sequence of strain DCY 17^T was found to be a continuous stretch of 1477 nt. The 16S rRNA gene sequences of related taxa were obtained from GenBank. Strain DCY 17^T and related strains were determined to have the highest sequence similarities to *Microbacterium esteraromaticum* DSM 8609^T (96.1% similarity to strain DCY 17^T), *M. xylanilyticum* DSM 16914^T (96.0%), *M. aquimaris* JS54-2^T (95.6%), *M. insulae* DS-66^T (95.5%), *M. ketosireducens* IFO 14548^T (95.5%), *M. arabinogalactanolyticum* DSM 8611^T (95.4%) and *M. halophilum* IFO 16062^T (94.6%). Strains Ms1 to Ms4 showed 95.7% similarity to *M. halophilum* IFO 16062^T. The similarity between these isolates was 99.0–99.5%. Sequence similarities of strains Ms1–Ms4 to strains of other *Microbacterium* species were similar to those of strain DCY 17^T (the 16S rRNA gene sequences of the five strains are almost identical). The phylogenetic tree (Fig. 1) showed that strains Ms1–Ms4 and DCY 17^T clearly belong to the genus *Microbacterium*.

The DNA G + C content of strain DCY 17^T was 70.2 mol% and those of strains Ms1–Ms4 were 68.9–73.5 mol% (Table 1). Strain DCY 17^T exhibited high levels of DNA–DNA relatedness with strains Ms1–Ms4 (>80%) and cross-hybridization values of strains Ms1–Ms4 also showed high levels of DNA relatedness (>80%; Supplementary Table S2). Thus, our results support the placement of strains DCY 17^T, Ms1, Ms2, Ms3 and Ms4 within a separate and previously unrecognized species, for which the name *Microbacterium soli* sp. nov. is proposed.

Description of *Microbacterium soli* sp. nov.

Microbacterium soli (so'li. L. neut. gen. n. *soli* of soil, the source of the first strains).

Gram-type-positive, aerobic, non-motile and rod-shaped (about 0.2–0.5 μ m wide and 0.5–0.9 μ m long) after growth on R2A agar (Difco) at 30 °C for 5 days. Colonies are circular and pale yellow on R2A agar after 5 days of incubation at 30 °C. Grows at 25–42 °C, with optimal growth at 30 °C on R2A agar. Oxidase-negative and catalase-positive. Produces acid phosphatase, cysteine arylamidase, α -galactosidase, α -glucosidase, β -galactosidase, β -glucosidase (aesculin hydrolysis), leucine arylamidase, α -mannosidase and valine arylamidase but does not produce arginine dihydrolase, α -fucosidase, β -glucuronidase, lipase (C14), protease (gelatin hydrolysis) and urease. Produces acid from cellobiose, aesculin, gluconate and trehalose and not from D-adonitol (ribitol), D- or L-arabitol, dulcitol (galactitol), erythritol, D- or L-fucose, glycerol, glycogen, inositol, inulin, 2-ketogluconate, D-lyxose, methyl α -D-mannoside, raffinose, L-rhamnose, sorbitol, L-sorbose, starch, D-tagatose, L-xylose or xylitol. Does not assimilate acetate, adipate, L-alanine, caprate, citrate, L-fucose, glycogen, L-histidine, 3-hydroxybenzoate, 3-hydroxy-DL-butyrate, inositol, itaconate, 2-ketogluco-

nate, 5-ketogluconate, DL-lactate, L-malate, malonate, melibiose, propionate, L-rhamnose, D-ribose, L-serine, sorbitol, suberate or n-valerate but does assimilate N-acetyl-D-glucosamine, L-arabinose, gluconate, glucose, maltose, mannitol, mannose, phenylacetate, L-proline, salicin and sucrose. The predominant quinones are MK-12, MK-11 and MK-13. Major cellular fatty acids are C_{15:0} anteiso, C_{15:0} iso, C_{16:0} iso, C_{17:0} anteiso, C_{17:0} iso and C_{14:0} iso. Does not reduce nitrate to nitrite or nitrogen gas. The cell-wall peptidoglycan is of the B2 β type, (L-Hsr)–D-Glu→Gly→D-Orn with glycolyl residues. Cell-wall sugars are galactose and traces of an unknown sugar. The DNA G + C contents of known strains are 68.9–73.5 mol%.

The type strain, DCY 17^T (=KCTC 19237^T =LMG 24010^T), was isolated from soil of a ginseng field in Daejeon, South Korea. Four other strains (Ms1–Ms4) were isolated from the same source.

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