Curtobacterium ginsengisoli sp. nov., isolated from soil of a ginseng field

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A Gram-positive, non-motile, pale-yellow, short rod-shaped bacterium, strain DCY26^T, was isolated from soil of a ginseng field in South Korea and was investigated to determine its taxonomic position. The organism grew optimally at 30–37 °C. The G+C content of its DNA was 65.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain DCY26^T was related most closely to species of the genus *Curtobacterium*, in the family *Microbacteriaceae*. Strain DCY26^T showed highest 16S rRNA gene sequence similarity to *Curtobacterium pusillum* DSM 20527^T (96.3 %), *Curtobacterium luteum* DSM 20542^T (96.2 %), *Curtobacterium flaccumfaciens* LMG 3645^T (96.2 %), *Curtobacterium citreum* DSM 20528^T (96.1 %), *Curtobacterium albidum* DSM 20512^T (96.1 %) and *Curtobacterium herbarum* DSM 14013^T (95.3 %). The predominant menaquinone of strain DCY26^T was MK-9. Other chemotaxonomic data also supported the affiliation of strain DCY26^T to the genus *Curtobacterium*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain DCY26^T is considered to represent a novel species of the genus *Curtobacterium*, for which the name *Curtobacterium ginsengisoli* sp. nov. is proposed. The type strain is DCY26^T (=KCTC 13163^T =JCM 14773^T).

The family *Microbacteriaceae*, proposed by Park *et al.* (1993), comprises more than 20 genera, including the genus *Curtobacterium*. The genus *Curtobacterium* was established by Yamada & Komagata (1972) to accommodate six species, namely *Curtobacterium albidum*, *C. citreum*, *C. luteum*, *C. pusillum*, *C. saperdae* and *C. testaceum*. Four other species were later added to the genus: *Curtobacterium flaccumfaciens* (Hedges 1922) Collins and Jones 1984, *C. plantarum* Dunleavy 1989, *C. herbarum* Behrendt *et al.* 2002 and *C. ammoniigenes* Aizawa *et al.* 2007. *C. saperdae* and *C. testaceum* were subsequently transferred to the genus *Microbacterium* (Takeuchi & Hatano, 1998), and *C. plantarum* was transferred to the genus *Pantoea* (Gavini *et al.*, 1989). At the time of writing, the genus *Curtobacterium* thus comprises seven recognized species.

In a series of studies, we attempted to isolate microorganisms from soil in order to investigate the community structure based on a culture-dependent method. In the present study, a *Curtobacterium*-like bacterium, designated strain DCY26^T, was isolated from soil of a ginseng field in Daejeon city, South Korea, and was characterized based on a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequences, determination of genomic relatedness and tests of chemotaxonomic and phenotypic properties were conducted to determine the precise taxonomic position of strain DCY26^T. The results indicate that strain DCY26^T represents a novel species of the genus *Curtobacterium*.

Strain DCY26^T was 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 a 100 µl aliquot was inoculated on ten-fold-diluted R2A agar (Difco). Single colonies on these agar plates were purified by transferring them onto new plates and subjecting them to an additional incubation for 5 days at 30 °C. The purified colonies were tentatively identified based on analysis of the partial 16S rRNA gene sequence.

Cell morphology and motility were observed with a Nikon light microscope (×1000 magnification), with the cells being allowed to grow for 5 days at 30 °C on R2A agar. Gram reactions were 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 based on measurement of bubble production following the application of 3 % (v/v) hydrogen peroxide solution. Growth at various temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar and growth at different pH values was assessed in R2A broth. Growth on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY26^T is EF587758.

nutrient agar, Luria–Bertani agar and trypticase soy agar was also evaluated at 30 °C. The API 20NE, API ID32 GN and API 32 ZYM microtest systems were employed according to the recommendations of the manufacturer (bioMérieux).

Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), purified via TLC and subsequently analysed by HPLC as described by Collins & Jones (1981) and Shin *et al.* (1996).

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

Genomic DNA was extracted and purified with the Genomic DNA isolation kit (Core Bio System). The 16S rRNA gene was amplified from chromosomal DNA by using the universal bacterial primer set 9F and 1512R (Weisburg *et al.*, 1991), and the purified PCR products were sequenced by Genotec (Daejeon, Korea) (Kim *et al.*, 2005). The full sequence of the 16S rRNA gene was compiled with the SeqMan software and 16S rRNA gene sequences of related taxa were obtained from GenBank and edited by using the BioEdit program (Hall, 1999). Multiple alignments were performed with the CLUSTAL_X program (Thompson *et al.*, 1997). Evolutionary distances were calculated by using Kimura's two-parameter model (Kimura, 1983). A phylogenetic tree was constructed via the neighbour-joining method (Saitou & Nei, 1987) in the MEGA 2 program (Kumar *et al.*, 2001). Bootstrap analysis based on 1000 replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). All recognized species of the genus *Curtobacterium* and related genera were included in the phylogenetic tree.

Strain DCY26^T was cultured on R2A agar at 30 °C, yielding pale-yellow, circular colonies. Cells were Gram-positive, non-motile, short rods. Strain DCY26^T was able to grow at 25–42 °C but not at 4 °C. Results relating to the physiological characteristics of strain DCY26^T are summarized in the species description, and a comparison of selective characteristics with the type strains of related species is provided in Table 1.

The major cellular fatty acids of strain DCY26^T were anteiso-$C_{15:0}$ (54 %), anteiso-$C_{17:0}$ (29 %), iso-$C_{16:0}$ (11 %) and iso-$C_{15:0}$ (4 %). This profile of major fatty acids is common to members of the genera *Curtobacterium* and *Rathayibacter*. Strain DCY26^T contained menaquinones with nine isoprene units (MK-9) as the predominant isoprenoid quinone, consistent with members of the genus *Curtobacterium* (Behrendt *et al.*, 2002). By contrast, species of the genus *Rathayibacter* possess MK-10 as the predominant menaquinone with a smaller amount (<20 %) of MK-9 (Suzuki *et al.*, 1997) (Table 1). Strain DCY26^T contained D-ornithine as the diamino acid in the peptidoglycan, which is commonly found in species of the genus *Curtobacterium*; members of the genus *Rathayibacter* possess L-diaminobutyric acid as the diamino acid in the peptidoglycan (Behrendt *et al.*, 2002).

The G+C content of the genomic DNA of strain DCY26^T was 65.8 mol%. This result was also consistent with data for the genus *Curtobacterium*.

The 16S rRNA gene sequence of strain DCY26^T was found to be a continuous stretch of 1390 nt. The 16S rRNA gene sequences of related taxa were obtained from GenBank. Sequence analysis indicated that strain DCY26^T belonged to the genus *Curtobacterium* within the family *Microbacteriaceae*. Strain DCY26^T showed highest levels of 16S rRNA gene sequence similarity to *C. pusillum* DSM 20527^T (96.3 %), *C. luteum* DSM 20542^T (96.2 %), *C. flaccumfaciens* LMG 3645^T (96.2 %), *C. citreum* DSM 20528^T (96.1 %), *C. albidum* DSM 20512^T (96.1 %) and *C. herbarum* DSM 14013^T (95.3 %). In the neighbour-joining phylogenetic tree (Fig. 1), strain DCY26^T clearly belonged to the lineage containing members of the genus *Curtobacterium*, as evidenced by the high bootstrap value. Based on 16S rRNA gene sequencing, the phylogenetic position of strain DCY26^T among members of the genus *Curtobacterium* was unique and distinct.

On the basis of phenotypic, chemotaxonomic and phylogenetic data, we concluded that strain DCY26^T represents a novel species of the genus *Curtobacterium*, for which the name *Curtobacterium ginsengisoli* sp. nov. is proposed.

Description of *Curtobacterium ginsengisoli* sp. nov.

Curtobacterium ginsengisoli (gin.sen.gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* of soil of a ginseng field, the source of the type strain).

Cells are Gram-positive, non-motile, short rods, 0.5–0.8 μm in length and 0.3–0.6 μm in diameter after growth on R2A agar at 30 °C for 5 days. Colonies grown on R2A agar plates for 5 days are pale yellow. Grows optimally at 30–37 °C. Growth is observed at 25–42 °C and at pH 5.0–11.0 but not at below 4 °C. Positive for oxidase and weakly positive for catalase. Production of indole and acid from glucose is negative. Produces *N*-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase, α-fucosidase, α-galactosidase, α-glucosidase, β-glucuronidase, β-galactosidase, β-glucosidase, leucine arylamidase, lipase (C14), naphthol-AS-BI-phosphohydrolase and valine arylamidase, but not arginine dihydrolase, α-mannosidase, protease, trypsin or urease. Assimilates L-arabinose, D-glucose, maltose, L-rhamnose, sucrose and inositol, but not 2-ketogluconate, 3-hydroxybenzoate, DL-3-hydroxybutyrate, 4-hydroxybenzoate, 5-ketogluconate, adipate, caprate, citrate, gluconate, itaconate, lactate, phenyl acetate, L-fucose, D-mannose, melibiose, D-ribose, D-mannitol, D-sorbitol, L-alanine, L-

Table 1. Differential phenotypic characteristics between strain DCY26^T and the type strains of related *Curtobacterium* and *Rathayibacter* species

Strains: 1, DCY26^T (data from the present study); 2, *C. albidum* DSM 20512^T (Aizawa *et al.*, 2007; Behrendt *et al.*, 2002); 3, *C. ammoniigenes* NBRC 101786^T (Aizawa *et al.*, 2007); 4, *C. citreum* DSM 20528^T (Aizawa *et al.*, 2007; Behrendt *et al.*, 2002); 5, *C. flaccumfaciens* LMG 3645^T (Aizawa *et al.*, 2007; Behrendt *et al.*, 2002); 6, *C. herbarum* DSM 14013^T (Behrendt *et al.*, 2002); 7, *C. luteum* DSM 20542^T (Aizawa *et al.*, 2007; Behrendt *et al.*, 2002); 8, *C. pusillum* DSM 20527^T (Aizawa *et al.*, 2007; Behrendt *et al.*, 2002); 9, *R. caricis* VKM Ac-1799^T (Dorofeeva *et al.*, 2002); 10, *R. festucae* VKM Ac-1390^T (Dorofeeva *et al.*, 2002); 11, *R. iranicus* DSM 7484^T (Behrendt *et al.*, 2002; Dorofeeva *et al.*, 2002); 12, *R. rathayi* DSM 7485^T (Behrendt *et al.*, 2002; Dorofeeva *et al.*, 2002); 13, *R. toxicus* DSM 7488^T (Behrendt *et al.*, 2002; Dorofeeva *et al.*, 2002; Sasaki *et al.*, 1998); 14, *R. tritici* DSM 7486^T (Behrendt *et al.*, 2002; Dorofeeva *et al.*, 2002). +, Positive; −, negative; ND, no data available. All strains were Gram-positive, catalase-positive and able to assimilate D-glucose. The following tests were negative for strain DCY26^T, *C. albidum* DSM 20512^T, *C. ammoniigenes* NBRC 101786^T, *C. citreum* DSM 20528^T, *C. flaccumfaciens* LMG 3645^T, *C. luteum* DSM 20542^T and *C. pusillum* DSM 20527^T (no data available for the other strains): nitrate reduction, urease activity and assimilation of DL-3-hydroxybutyrate, propionate, L-histidine and serine.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Motility	−	−	−	+	−	+	+	+	−	−	−	−	−	−
Cell morphology	Short rods	ND	Rods	ND	ND	Rods	ND	ND	Rods	Rods	ND	ND	Rods	ND
Colony colour	Yellow	Ivory	Yellow	Yellow	Yellow	Orange	Yellow	Yellow	Yellow	Orange	Yellow	Yellow	Yellow	Yellow
Oxidase	+	−	−	−	−	−	−	−	W	+	W	W	W	W
Growth at 4 °C	−	W	−	W	W	W	W	W	−	−	W	W	W	W
Production of acid from glucose	−	+	ND	+	+	+	+	+	ND	ND	+	+	+	+
Enzyme activity														
α-Glucosidase	+	−	+	+	+	+	+	+	−	−	−	−	−	−
β-Galactosidase	+	−	+	+	+	ND	+	−	ND	ND	ND	ND	ND	ND
β-Glucosidase	+	+	+	+	+	+	−	+	−	−	+	W	−	+
Protease	−	+	−	−	+	+	−	+	ND	ND	−	−	−	−
Assimilation of:														
Citrate	−	−	−	−	−	ND	−	−	−	+	ND	ND	ND	ND
Gluconate	−	+	+	−	+	ND	−	+	ND	+	ND	ND	ND	ND
Lactate	−	−	+	W	+	ND	W	−	−	ND	ND	ND	ND	ND
L-Arabinose	+	+	−	+	+	+	+	+	+	+	+	+	+	+
L-Fucose	−	−	−	+	W	ND	+	−	−	ND	ND	ND	ND	ND
Melibiose	−	+	+	+	+	+	+	+	+	+	−	−	−	−
L-Rhamnose	+	+	−	+	+	+	+	+	+	+	ND	ND	ND	ND
D-Ribose	−	+	ND	+	+	+	+	+	−	−	ND	ND	ND	ND
D-Mannitol	−	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Sorbitol	−	−	ND	−	−	+	−	−	+	+	−	−	+	+
L-Alanine	−	W	−	−	+	ND	−	−	ND	ND	ND	ND	ND	ND
L-Proline	−	−	−	−	+	ND	−	−	+	+	ND	ND	ND	ND
N-Acetyl-D-glucosamine	−	W	−	+	+	ND	+	−	ND	ND	ND	ND	ND	ND
Glycogen	−	−	−	+	W	ND	+	−	ND	ND	ND	ND	ND	ND
Predominant menaquinone	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9	MK-10	MK-10	MK-10	MK-10	MK-10	MK-10
DNA G+C content (mol%)	65.8	ND	68.8	ND	ND	71	ND	ND	68.4	68.2	ND	ND	60.4	ND

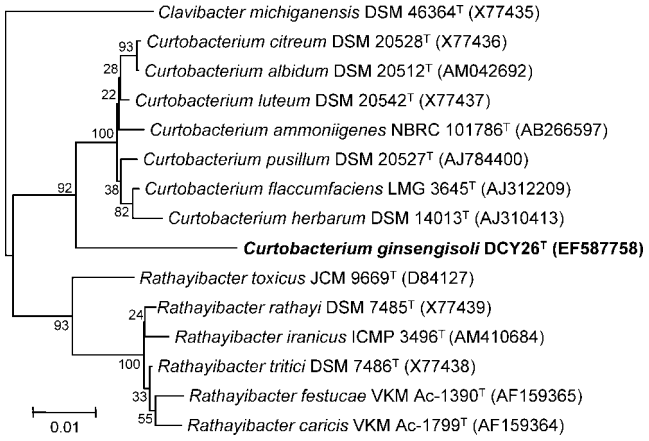

Fig. 1. Neighbour-joining phylogenetic tree showing the relationships among strain DCY26^T, *Curtobacterium* species and members of related genera based on 16S rRNA gene sequences. Bootstrap values are expressed as percentages of 1000 replications at branch points. Bar, 0.01 substitutions per nucleotide position.

histidine, L-proline, L-serine, *N*-acetyl-D-glucosamine, propionate, suberate, *n*-valerate, salicin or glycogen. Does not reduce nitrate to nitrite or to nitrogen gas. The predominant menaquinone is MK-9. The DNA G+C content of the type strain is 65.8 mol%, as determined by HPLC.

The type strain, DCY26^T (=KCTC 13163^T =JCM 14773^T), was isolated from soil of a ginseng field in South Korea.

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