Castellaniella ginsengisoli sp. nov., a β -glucosidase-producing bacterium

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A Gram-negative, motile bacterium, designated DCY36^T, was isolated from soil of a ginseng field in South Korea and was characterized using a polyphasic taxonomic approach. Comparative 16S rRNA gene sequence analysis showed that strain DCY36^T belongs to genus *Castellaniella* in the family *Alcaligenaceae* of the class *Betaproteobacteria*. The 16S rRNA gene sequence similarities between strain DCY36^T and the three recognized representatives of the genus, *Castellaniella caeni* Ho-11^T, *Castellaniella defragrans* 54Pin^T and *Castellaniella denitrificans* NKNTAU^T, were 98.4, 97.5 and 98.1 %, respectively. Strain DCY36^T exhibited relatively low levels of DNA–DNA relatedness with respect to these three species. The G + C content of the genomic DNA was 63.7 mol%. Strain DCY36^T contained ubiquinone Q-8. The major fatty acids were C_{16:0} (27.4 %), C_{18:1 ω 7c} (16.9 %) and summed feature 4 (C_{16:1 ω 7c} and C_{15:0} iso 2-OH, 32.5 %). On the basis of phenotypic and genotypic properties and phylogenetic distinctiveness, strain DCY36^T (=KCTC 22398^T=JCM 15515^T) should be classified in the genus *Castellaniella* as the type strain of a novel species, for which the name *Castellaniella ginsengisoli* sp. nov. is proposed.

The genus *Castellaniella* was created by the reclassification of *Alcaligenes defragrans* DSM 12141^T (Foss *et al.*, 1998) to the type species of the genus *Castellaniella* (Kämpfer *et al.*, 2006). At the time of writing, the genus contains three species, *Castellaniella defragrans* (Kämpfer *et al.*, 2006), *Castellaniella denitrificans* (Kämpfer *et al.*, 2006) and *Castellaniella caeni* (Liu *et al.*, 2008).

In the course of screening micro-organisms obtained from the soil of a ginseng field, a Gram-negative and motile strain, DCY36^T, was isolated. On the basis of 16S rRNA gene sequence analysis, strain DCY36^T was considered to be a *Castellaniella*-like strain. Strain DCY36^T was subjected to a polyphasic taxonomic investigation and the results indicated that strain DCY36^T should be placed in a novel species in the genus *Castellaniella*.

Strain DCY36^T was isolated from surface soil of an agricultural field where ginseng was planted. Soil (1 g) was immersed in 50 ml saline (0.85 % NaCl) solution and vortexed. Aliquots (100 μ l) of serial dilutions were used to inoculate 1/10 R2A agar (Difco). Single colonies on the plates were purified by subculture under the same conditions. The isolate was routinely cultured on Luria–Bertani (LB) agar (Difco) at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C. *C. caeni* KCTC 12197^T, *C. defragrans* DSM 12141^T and *C. denitrificans* KCTC 22035^T obtained from the Korean Collection for Type Cultures (KCTC) and the German Collection of Microorganisms and Cell Cultures (DSMZ), were used as reference strains for DNA–DNA hybridization and other experiments.

The Gram reaction was performed by using the non-staining method, as described by Buck (1982). Cell morphology was examined by light microscopy (Eclipse E600; Nikon) and transmission electron microscopy (LEO 912 AB; Carl Zeiss), which was performed as follows. Bacteria were grown on LB agar at 30 °C for 18 h. Cells were gently resuspended in one drop of deionized H₂O and samples were placed on carbon- and Formvar-coated nickel grids for 30 s. Grids were floated on one drop of 0.1 % (w/v) aqueous uranyl acetate, blotted dry and viewed at

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Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCY36^T is EU873313.

Transmission electron micrographs of negatively stained cells of strain DCY36^T are available as supplementary figures with the online version of this paper.

100 kV under standard operating conditions. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Physiological characteristics were determined with API 20E, API 20NE, ID 32 GN and API ZYM galleries (bioMérieux) according to the manufacturer's instructions. Growth at different temperatures (4, 10, 15, 25, 30, 37 and 42 °C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed in LB broth after incubating for 5 days. Growth at 30 °C on nutrient agar, TSA and LB agar was also evaluated.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified by TLC and subsequently analysed by HPLC, as described by Collins & Jones (1981) and Shin *et al.* (1996). In order to perform fatty acid methyl ester analysis, cells were cultured on TSA at 30 °C for 48 h and two loops of cells were harvested. Fatty acid methyl esters were prepared, separated and identified with the Sherlock Microbial Identification System (MIDI) (Sasser, 1990). Polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1977).

For the phylogenetic analysis of strain DCY36^T, genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent) and the 16S rRNA gene was amplified by PCR. The purified PCR product was sequenced according to Kim *et al.* (2005) and the nearly complete sequence was compiled using SEQMAN software (DNASTAR). 16S rRNA gene sequences of related taxa were obtained from GenBank and edited using the BioEdit program (Hall, 1999). Multiple alignments were performed with CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) and phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in MEGA3 (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

For the determination of the genomic DNA G + C content, DNA was extracted and purified with the QIAGEN Genomic-tip system (100/G; Qiagen) and then enzymically degraded 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 micro-dilution wells. Five replicate hybridizations were conducted for each sample and the highest and lowest values from each sample were excluded. The remaining three values were utilized in the calculation of mean DNA–DNA relatedness values.

Strain DCY36^T was Gram-negative and non-spore-forming and cell morphology showed motile rods of 0.7–0.8 µm wide and 1.6–2.5 µm long (see Supplementary Fig. S1, available in IJSEM Online). The novel strain grew well at 37 °C and growth occurred at 42 °C. The phenotypic and chemotaxonomic characteristics that differentiate strain

DCY36^T from the other species in the genus *Castellaniella* are listed in Table 1.

The fatty acid profile of strain DCY36^T is compared with the profiles of other species of the genus *Castellaniella* in Table 2. These four species contained C_{16:0}, summed feature 4 (C_{16:1}ω7c and C_{15:0} iso 2-OH) and C_{18:1}ω7c as the predominant fatty acids. However, strain DCY36^T contained a higher content of summed feature 4. Strain DCY36^T contained ubiquinone Q-8 as the major respiratory quinone, which is a common characteristic of members of the class *Betaproteobacteria*.

Table 1. Differential phenotypic characteristics of strain DCY36^T and other species of the genus *Castellaniella*

Strains: 1, DCY36^T; 2, *C. caeni* KCTC 12197^T; 3, *C. defragrans* DSM 12141^T; 4, *C. denitrificans* KCTC 22035^T. All enzyme activities were analysed in this study. Other data in columns 2–4 were taken from Liu *et al.* (2008). All strains were positive for acid phosphatase, esterase (C4), esterase (C8), β-glucosidase (aesculin hydrolysis), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. All strains were negative for N-acetyl-β-glucosaminidase, arginine dihydrolase, α-chymotrypsin, α-galactosidase, β-galactosidase (ONPG hydrolysis), β-galactosidase (PNPG hydrolysis), α-glucosidase (starch hydrolysis), β-glucosidase (6-Br-2-naphthyl-β-D-glucopyranoside), β-glucuronidase, lipase (C14), protease (gelatin hydrolysis), trypsin and urease. +, Positive; –, negative; w, weak; ND, no data available.

Characteristic	1	2	3	4
Motility	+	–	+	+
Enzyme activities				
Alkaline phosphatase	+	–	–	+
Cystine arylamidase	–	–	w	–
α-Fucosidase	w	w	–	–
α-Mannosidase	–	w	–	–
Valine arylamidase	w	–	w	w
Assimilation of:				
N-Acetyl-D-glucosamine	+	–	–	–
Adipate	+	+	–	–
L-Arabinose	+	–	–	–
Citrate	+	–	+	+
L-Fucose	–	+	–	–
Gluconate	+	–	+	+
D-Glucose	+	+	–	–
L-Histidine	–	+	–	–
3-Hydroxybenzoate	+	–	+	+
4-Hydroxybenzoate	+	–	+	+
Itaconate	–	–	–	+
D-Mannose	+	–	–	–
D-Mannitol	+	–	–	–
Phenyl acetate	+	w	w	–
L-Serine	+	+	+	–
Suberate	–	+	–	–
DNA G + C content (mol%)	63.7	63.5	66.9	ND

Table 2. Fatty acid contents of strain DCY36^T and members of the genus *Castellaniella*

Strains: 1, DCY36^T; 2, *C. caeni* KCTC 12197^T; 3, *C. defragrans* DSM 12141^T; 4, *C. denitrificans* KCTC 22035^T. Only fatty acids comprising >1% of the total are shown. tr, Less than 1%.

Fatty acid (%)	1	2	3	4
Saturated				
C _{12:0}	5.9	8.4	6.4	5.4
C _{16:0}	27.4	23.5	35.0	31.2
C _{17:0}	tr	tr	1.4	tr
C _{17:0} cyclo	4.3	8.5	32.0	9.6
C _{18:0}	tr	tr	3.7	tr
C _{19:0} cyclo ω8c	tr	1.2	7.5	tr
Unsaturated				
C _{18:1} ω7c	16.9	19.3	1.8	14.0
Summed features*				
3	8.9	12.2	7.8	8.4
4	32.5	23.1	3.4	27.2

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1} iso I and C_{14:0} 3-OH. Summed feature 4 contained C_{16:1} ω7c and C_{15:0} iso 2-OH.

Strain DCY36^T exhibited an almost-identical polar lipid profile to those of other species of the genus *Castellaniella* (Kämpfer *et al.*, 2006), containing a predominance of phosphatidylethanolamine (PE) and moderate amounts of phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) and a minor amount of an unknown aminolipid. These observations are also in good agreement with the polar lipid composition of other members of the class *Betaproteobacteria* (Busse & Auling, 1988; Auling *et al.*, 1991). The predominance of PE over PG and DPG is characteristic for species of the genus *Castellaniella* (Stolz *et al.*, 2005). The polar lipid composition supported the

other data suggesting that strain DCY36^T belongs to the genus *Castellaniella*.

A nearly complete 16S rRNA gene sequence of strain DCY36^T (1441 bp) was obtained. On the basis of 16S rRNA gene sequence similarity, the closest relatives were *C. caeni* Ho-11^T (98.4%), *C. denitrificans* NKNTAU^T (98.1%), *C. defragrans* 54Pin^T (97.5%) and *Pusillimonas noertemannii* BN9^T (95.4%). A phylogenetic tree constructed with the neighbour-joining method using 16S rRNA gene sequences from strain DCY36^T and closely related species of the class *Betaproteobacteria* is shown in Fig. 1.

The DNA G+C content of strain DCY36^T is 63.7 mol%. To differentiate strain DCY36^T from other species of the genus *Castellaniella*, DNA–DNA hybridizations were performed. Strain DCY36^T exhibited relatively low levels of DNA–DNA relatedness with respect to *C. caeni* KCTC 12197^T (16.9%), *C. defragrans* DSM 12141^T (62.3%) and *C. denitrificans* KCTC 22035^T (22.3%), indicating that the novel strain was not related to them at the species level (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994).

The results of this polyphasic study clearly show that strain DCY36^T represents a novel species within the genus *Castellaniella*, for which the name *Castellaniella ginsengisoli* sp. nov. is proposed.

Description of *Castellaniella ginsengisoli* sp. nov.

Castellaniella 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 organism).

Cells are Gram-negative, motile and rod-shaped, 0.7–0.8 µm in width and 1.6–2.5 µm in length. After 3 days on R2A, colonies are 1–2 mm in diameter, smooth, yellow-coloured, circular and convex. Oxidase- and catalase-positive. Growth occurs on R2A agar, nutrient agar, TSA and LB agar at 25–42 °C (optimum 30 °C in LB broth) and at pH 5.0–9.0 (optimum pH 6.5–7.0 in LB broth). Reduces

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences (1391 bp) constructed with the neighbour-joining method, showing the phylogenetic relationships between strain DCY36^T and closely related species selected from the class *Betaproteobacteria*. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Burkholderia cepacia* ATCC 24516^T was used as the outgroup. Bar, 0.01 substitutions per nucleotide position.

nitrate to nitrogen gas. Positive for acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), β -glucosidase (aesculin hydrolysis), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. Negative for *N*-acetyl- β -glucosaminidase, arginine dihydrolase, α -chymotrypsin, cystine arylamidase, lipase (C14), α -galactosidase, β -galactosidase (ONPG), β -galactosidase (PNPG), α -glucosidase (starch hydrolysis), β -glucosidase (6-Br-2-naphthyl- β -D-glucopyranoside), β -glucuronidase, α -mannosidase, protease (gelatin hydrolysis), trypsin and urease. Utilizes 3- and 4-hydroxybenzoate, DL-3-hydroxybutyrate, acetate, adipate, caprate, citrate, gluconate, lactate, L-malate, phenylacetate, propionate, n-valerate, L-arabinose, D-glucose, maltose, D-mannose, D-mannitol, L-alanine, L-proline, L-serine and *N*-acetyl-D-glucosamine. Does not utilize 2- or 5-ketogluconate, itaconate, malonate, suberate, L-fucose, melibiose, L-rhamnose, D-ribose, sucrose, inositol, D-sorbitol, L-histidine, salicin or glycogen. The major fatty acids are C_{16:0}, C_{18:1} ω 7c and summed feature 4. The polar lipids detected are PE, DPG and PG.

The type strain, DCY36^T (=KCTC 22398^T=JCM 15515^T), was isolated from soil of a ginseng field in Daejeon, South Korea. The DNA G+C content of the type strain is 63.7 mol%.

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