Nocardioides panacihumi sp. nov., isolated from soil of a ginseng field

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A novel bacterial strain designated Gsoil 616^T was isolated from a soil sample of a ginseng field in Pocheon province (South Korea) and was characterized taxonomically by using a polyphasic approach. The isolate was Gram-positive, strictly aerobic, non-motile, non-spore-forming and rod- or coccoid-shaped. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate belongs to the genus *Nocardioides* in the family *Nocardioideaceae* but was clearly separated from established species of this genus. The 16S rRNA gene sequence similarities between strain Gsoil 616^T and the type strains of *Nocardioides* species with validly published names ranged from 91.8 to 96.1 %. The G + C content of the genomic DNA was 73 mol%. Phenotypic and chemotaxonomic data [major menaquinone MK-8(H₄) and major fatty acid iso-C_{16:0}] supported the affiliation of strain Gsoil 616^T to the genus *Nocardioides*. However, the results of physiological and biochemical tests allowed phenotypic differentiation of the isolate from other *Nocardioides* species. Therefore, strain Gsoil 616^T represented a novel species within the genus *Nocardioides*, for which the name *Nocardioides panacihumi* sp. nov. is proposed. The type strain is Gsoil 616^T (=KCTC 19187^T =DSM 18660^T).

The genus *Nocardioides* was proposed by Prauser (1976) and, at the time of writing, it comprises 20 recognized species, including the recently described species *Nocardioides furvisabuli* (Lee, 2007) and *Nocardioides insulae* (Yoon *et al.*, 2007). Strain Gsoil 616^T was isolated from soil of a ginseng field in Pocheon province, South Korea, during the study of bacterial populations and was tentatively identified as *Nocardioides* sp. (Im *et al.*, 2005). The isolation procedure and medium were described by Cui *et al.* (2007). Strain Gsoil 616^T was routinely cultured on R2A agar (Difco) or half-strength R2A agar at 30 °C and maintained in glycerol suspension (20 %, w/v) at –70 °C.

The Gram reaction was performed by the non-staining method as described by Buck (1982). Cell morphology was observed at ×1000 magnification with a light microscope (Nikon) using cells grown for 3 days at 30 °C on R2A agar. Motility was tested using the hanging drop technique. Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Carbon-source utilization and enzyme activities were tested by using the

API 20 NE, API ID 32 GN, API 50 CH and API ZYM test kits (bioMérieux). Anaerobic growth was tested in serum bottles with sodium thioglycolate (1 g l^{–1}) added to R2A broth and replacing the upper air layer with nitrogen gas. Tests for the degradation of DNA [with DNase agar (Scharlau); performed by flooding plates with 1 M HCl], casein, chitin, starch (Atlas, 1993), xylan and cellulose (Ten *et al.*, 2004) were evaluated after 2 weeks. Growth at different temperatures (4, 15, 20, 25, 30, 37 and 42 °C) and pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed after 5 days incubation. Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation. Growth on nutrient agar, trypticase soy agar (TSA) and MacConkey agar was also evaluated, at 30 °C.

Cells of strain Gsoil 616^T were strictly aerobic, Gram-positive, non-motile, non-spore-forming and rod- or coccoid-shaped. Colonies grown on R2A agar plates for 3 days were smooth, circular, white and 1–3 mm in diameter. The isolate grew well on nutrient agar and TSA, whereas it could not grow on MacConkey agar. Other physiological characteristics of strain Gsoil 616^T are summarized in the species description. Phenotypic characteristics that differentiate the isolate from its closest phylogenetic relatives are listed in Table 1.

The study of DNA G + C content and chemotaxonomic characters was carried out as described previously (Cui

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 616^T is AB271053.

A comparison of the fatty acid profile of strain Gsoil 616^T and related type strains is available as supplementary material with the online version of this paper.

Table 1. Comparison of the phenotypic characteristics of strain Gsoil 616^T and related species of genus *Nocardioides*

Strains/species: 1, Gsoil 616^T; 2, *Nocardioides luteus*; 3, *Nocardioides aquiterrae* GW-9^T; 4, *Nocardioides pyridinolyticus* OS4^T; 5, *Nocardioides albus* (data in parentheses are for the type strain). Data for reference taxa were taken from Yoon *et al.* (2006). All taxa were positive for utilization of D-fructose, D-glucose, sucrose and D-xylose, hydrolysis of gelatin and leucine arylamidase and trypsin activities and negative for hydrolysis of urea and lipase (C₁₄), α -galactosidase and α -chymotrypsin activities. +, Positive; –, negative; v, variable reaction; w, weakly positive reaction; ND, no data available.

Characteristic	1	2	3	4	5
Cell morphology	Short rods, cocci	Hyphae	Rods, cocci	Rods, cocci	Hyphae
Cell size (µm)	0.3–0.5 × 0.7–1.2	0.5–1.0*	0.8–1.0 × 1.7–2.0	0.5–0.6 × 1.2–1.6	0.5–1.0*
Motility	–	–	+	+	–
Colony colour	White	Yellow to orange-yellow or cream	Cream	Cream	Whitish to faintly brownish
Nitrate reduction	+	–	+	+	(–)
Hydrolysis of:					
Aesculin	+	+	+	+	(w)
Starch	–	+	–	+	(+)
Utilization as sole carbon and energy sources of:					
D-Cellobiose	–	+	+	+	(+)
D-Galactose	–	–	+	+	(+)
D-Mannitol	–	+	+	+	+
D-Mannose	–	+	+	+	(+)
D-Raffinose	+	–	–	–	v (–)
Trehalose	–	ND	+	+	ND
API ZYM tests					
Alkaline phosphatase	+	v	–	+	(+)
Esterase (C4)	+	+	–	–	(+)
Valine arylamidase	w	–	–	w	(–)
Cystine arylamidase	w	–	w	–	(–)
Acid phosphatase	+	–	+	+	(–)
β -Galactosidase	–	v	–	–	(v)
α -Glucosidase	+	v	+	+	(+)
β -Glucosidase	+	–	w	–	(w)
α -Mannosidase	+	–	w	–	(w)
Isolation source	Soil	Soil	Groundwater	Oil-shale column	Soil
DNA G + C content (mol%)	73	68	73	73	67

*Cell width is shown.

et al., 2007). The G + C content of genomic DNA of strain Gsoil 616^T was 73 mol%. Cellular fatty acids were determined in a culture grown on R2A agar for 6 days. The fatty acid profile of strain Gsoil 616^T (compared with those of related type strains in Supplementary Table S1 in IJSEM Online) was composed of branched-chain fatty acids iso-C_{16:0} (45.5%), iso-C_{15:0} (3.8%), iso-C_{14:0} (2.9%), iso-C_{15:0} 3-OH (1.5%), iso-C_{17:0} (0.9%), ante-iso-C_{15:0} (0.8%), ante-iso-C_{17:0} (0.8%) and iso-C_{18:0} (0.5%), unsaturated fatty acids C_{17:1}ω6c (7.5%), C_{17:1}ω8c (3.6%), C_{18:1}ω9c (2.8%) and C_{18:3}ω6c (6,9,12) (2.1%), saturated fatty acids C_{16:0} (6.4%), C_{17:0} (5.9%), C_{18:0} (4.4%), C_{15:0} (1.6%) and C_{14:0} (0.9%), 10-methyl fatty acids C_{17:0} (2.8%), C_{16:0} (1.0%) and C_{18:0} (0.9%) and summed feature 7 (any combination of C_{18:1}ω7c, C_{18:1}ω9t and C_{18:1}ω12t) (1.4%), summed feature 3 (iso-C_{15:0} 2-OH and/or C_{16:1}ω7c) (1.1%) and

summed feature 6 (C_{18:2}ω6,9c and/or ante-iso-C_{18:0}) (0.4%). This fatty acid profile, including the major fatty acid iso-C_{16:0} and three 10-methyl fatty acids, is similar to those of *Nocardioides* species, although there are differences in the proportions of some fatty acids, perhaps because of differences in the extraction and cultivation conditions (Yoon *et al.*, 1997, 2004; Yi & Chun, 2004a, b). LL-Diaminopimelic acid and the predominant menaquinone MK-8(H₄) were also typical of members of the genus *Nocardioides* (Yoon *et al.*, 1997, 2004; Yi & Chun, 2004a, b).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to Kim *et al.* (2005). The full sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank.

Multiple alignments were performed by using the CLUSTAL_X program (Thompson *et al.*, 1997) and gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) and phylogenetic trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987) using the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

The nearly complete 16S rRNA gene sequence (1457 bp) of strain Gsoil 616^T was determined and subjected to phylogenetic analysis, which indicated that strain Gsoil 616^T belongs to the genus *Nocardioides* (Fig. 1). Strain Gsoil 616^T showed highest 16S rRNA gene sequence similarity to *Nocardioides luteus* KCTC 9575^T and *Nocardioides aquiterrae* GW-9^T (96.1 %), followed by *Nocardioides pyridinolyticus* KCTC 0074BP^T (95.5 %) and other species of *Nocardioides* (<95 %). These values (<97 %) were low enough to place strain Gsoil 616^T within a novel species of the genus *Nocardioides* according to Stackebrandt & Goebel (1994).

On the basis of the data described above, strain Gsoil 616^T should be assigned to the genus *Nocardioides* as the type strain of a novel species, for which the name *Nocardioides panacihumi* sp. nov. is proposed.

Description of *Nocardioides panacihumi* sp. nov.

Nocardioides panacihumi (pa.na.ci.hu'mi. N.L. n. *Panax* scientific name of ginseng; N.L. gen. n. *panacis* of ginseng; L. n. *humus* soil; N.L. gen. n. *panacihumi* of soil of a ginseng field).

Cells are Gram-positive, strictly aerobic and non-motile, 0.3–0.5 µm in diameter and 0.7–1.2 µm long after 3 days on R2A agar. Cells show rod-to-coccus morphogenesis from the early exponential phase to the stationary phase. Colonies grown on R2A agar (Difco) for 3 days are

smooth, circular, convex, opaque and white. Neither substrate mycelium nor aerial mycelium is formed. Good growth occurs at 30 °C and at pH 7.0. Growth is observed at 15–30 °C, pH 5.0–8.0 and in 0–1 % (w/v) NaCl. Nitrate is reduced weakly under aerobic conditions. Anaerobic growth does not occur. DNA and casein are degraded but xylan, cellulose, starch and chitin are not. Substrate utilization, enzyme production and other physiological characteristics are indicated in Table 1. In addition to the properties listed in Table 1, acetate, L-alanine, gluconate, glycogen, 3-hydroxybenzoate, 4-hydroxybenzoate, 3-hydroxybutyrate, maltose, phenylacetate, L-proline, propionate, L-rhamnose, salicin and valerate are utilized as sole carbon sources. *N*-Acetyl-β-glucosamine, adipate, L-arabinose, caprate, citrate, L-fucose, L-histidine, inositol, itaconate, 2-ketogluconate, 5-ketogluconate, L-lactate, malate, malonate, mannitol, D-melibiose, D-ribose, L-serine, D-sorbitol and suberate are not utilized as sole carbon sources. In API 50 CH tests, acid is produced from amygdalin, L-arabinose, aesculin ferric citrate, D-glucose, D-fructose, D-lyxose, D-raffinose, L-rhamnose, sucrose and D-turanose, but acid is not produced from *N*-acetylglucosamine, L-arabinose, DL-arabitol, arbutin, adonitol, starch, D-cellobiose, dulcitol, erythritol, DL-fucose, D-galactose, gentiobiose, glycerol, glycogen, methyl α-glucopyranoside, gluconate, inositol, inulin, 2- or 5-ketogluconate, D-lactose, D-lyxose, maltose, D-mannitol, D-mannose, methyl α-D-mannoside, melezitose, melibiose, ribose, salicin, D-sorbitol, L-sorbose, D-tagatose, trehalose, xylitol, L-xylose or methyl β-D-xyloside. According to the API ZYM gallery, the type strain is positive for *N*-acetyl-β-glucosaminidase, esterase lipase (C8) and α-fucosidase and negative for β-glucuronidase and naphthol-AS-BI-phosphohydrolase. According to the API 20NE gallery, the type strain is positive for β-glucosidase but negative for arginine dihydrolase, β-galactosidase, tryptophan degradation and glucose fermentation. The diagnostic diamino acid in the cell-wall peptidoglycan is LL-2,6-diaminopimelic acid. The

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain Gsoil 616^T with related species. The tree was made using the neighbour-joining method (Saitou & Nei, 1987) with a two-parameter distance matrix (Kimura, 1983) and pairwise deletion. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

predominant menaquinone is MK-8(H₄). The major fatty acid (>45 % of total fatty acids) is iso-C_{16:0}. The G+C content of genomic DNA of the type strain is 73 mol%. Other phenotypic characteristics are given in Table 1.

The type strain, Gsoil 616^T (=KCTC 19187^T =DSM 18660^T), was isolated from soil of a ginseng field of Pocheon province, South Korea.

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