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

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A Gram-positive, rod-shaped, non-spore-forming bacterium (Gsoil 346^T) was isolated from the soil of a ginseng field in South Korea and was characterized in order to determine its taxonomic position. On the basis of 16S rRNA gene sequences, strain Gsoil 346^T was shown to belong to the genus *Nocardioides* in the family *Nocardioideaceae*, with the most closely related species being *Nocardioides aquiterrae* GW-9^T (96.6% 16S rRNA gene sequence similarity); however, the strain clustered in a distinct branch of the phylogenetic tree with *Nocardioides kongjuensis* A2-4^T (96.2%), *Nocardioides aromaticivorans* H-1^T (96.1%), *Nocardioides nitrophenolicus* NSP41^T (96.1%) and *Nocardioides simplex* ATCC 15799^T (95.9%). Strain Gsoil 346^T was characterized chemotaxonomically and found to have LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan, phosphatidylinositol and phosphatidylglycerol as the major polar lipids, MK-8(H₄) as the predominant menaquinone and iso-C_{16:0}, C_{18:1ω9c} and C_{17:1ω8c} as the major fatty acids. The G+C content of the genomic DNA of the novel strain was 73.0 mol%. These chemotaxonomic properties supported the placement of strain Gsoil 346^T in the genus *Nocardioides*. The results of physiological and biochemical tests, along with the phylogenetic analysis, allowed strain Gsoil 346^T to be differentiated genotypically and phenotypically from recognized species of the genus *Nocardioides*. Therefore, strain Gsoil 346^T represents a novel species, for which the name *Nocardioides panacisoli* sp. nov. is proposed, with Gsoil 346^T (=KCTC 19470^T=DSM 21348^T) as the type strain.

The genus *Nocardioides* was proposed by Prauser (1976) and, at the time of writing, comprised 37 species with validly published names. The genus has been expanding rapidly in recent years: seven novel species were reported in 2007 and nine novel species were reported in 2008, including *Nocardioides agariphilus* (Dastager *et al.*, 2008), *Nocardioides daphniae* (Tóth *et al.*, 2008) and *Nocardioides hwasunensis* (Lee *et al.*, 2008). The species in this genus have been isolated from diverse habitats. For example, *Nocardioides aestuarii* (Yi & Chun, 2004) and *Nocardioides salarii* (Kim *et al.*, 2008) were isolated from marine sources, *Nocardioides panacihumi* (An *et al.*, 2007) and *Nocardioides kongjuensis* (Yoon *et al.*, 2006) were isolated from terrestrial sources and *Nocardioides fonticola* (Chou

et al., 2008) was isolated from a freshwater spring. Importantly, some species in the genus have been isolated from environments that were polluted with organic compounds, e.g. *Nocardioides aromaticivorans* (Kubota *et al.*, 2005) and *Nocardioides oleivorans* (Schippers *et al.*, 2005).

During a study of the culturable aerobic bacterial community of the soil from a ginseng field in Pocheon province, South Korea, a large number of novel bacterial strains were isolated (Im *et al.*, 2005). One of these isolates, strain Gsoil 346^T, was identified as a member of the genus *Nocardioides* in the family *Nocardioideaceae*. To determine the precise taxonomic designation of this strain, we conducted a phylogenetic analysis using 16S rRNA gene sequences and chemotaxonomic and phenotypic tests were used to determine important characteristics.

Strain Gsoil 346^T was isolated from the soil of a ginseng field in Pocheon province in South Korea (37° 58' N 127° 15' E). Soil was well suspended with 50 mM pot-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 346^T is FJ666101.

A supplementary figure showing the polar lipid profile of strain Gsoil 346^T and a table detailing the cellular fatty acid profiles of strain Gsoil 346^T and related type strains are available as supplementary material with the online version of this paper.

assium phosphate buffer (pH 7.0) and serial dilutions were spread on modified R2A agar (per litre: 0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g Casamino acids, 0.25 g soytone, 0.5 g glucose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4 , 0.05 g $MgSO_4$, 0.05 g $CaCl_2$, 15 g agar, pH 7.2). The plates were incubated at 30 °C for 1 month under aerobic conditions. Single colonies were subcultured on modified R2A agar and incubated at 30 °C for 1 week. The identities of the purified colonies were tentatively determined by sequencing a portion of the 16S rRNA gene (Im *et al.*, 2005). Strain Gsoil 346^T was one of the predominant isolates. The strain was subsequently routinely cultured on R2A agar (Difco; Reasoner & Geldreich, 1985) at 30 °C and preserved as a glycerol suspension (20 %, v/v) at -70 °C.

The Gram-reaction was determined by the non-staining method as described by Buck (1982). Morphology of cells after growth at 30 °C for 4 days on R2A agar was observed under a phase-contrast microscope (Optiphot-2, Nikon) at ×1000 magnification. Catalase activity was determined by bubble production in 3 % (v/v) H_2O_2 and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. For single-carbon-source assimilation studies, a defined liquid medium containing basal salts was used (per litre: 1.8 g K_2HPO_4 , 1.08 g KH_2PO_4 , 0.5 g $NaNO_3$, 0.5 g NH_4Cl , 0.1 g KCl , 0.1 g $MgSO_4$, 0.05 g $CaCl_2$), with the addition of vitamin solution (Widdel & Bak, 1992), trace element solution SL-10 (Widdel *et al.*, 1983) and selenite/tungstate solution (Tschech & Pfennig, 1984) and the pH adjusted to pH 6.8. This liquid medium was poured into 96-well plates (0.25 ml per well) and filter-sterilized carbon sources (0.1 % final concentration, w/v) were added into each well. The plates were incubated at 30 °C and growth was examined visually for up to 7 days. Negative control wells did not contain any of the carbon sources and positive control wells contained R2A broth. Some physiological characteristics were determined with API 20E and API ZYM galleries (bioMérieux) according to the manufacturer's instructions. Degradation of DNA [using DNase agar (Scharlau), supplemented with 1 M HCl], degradation of casein, chitin and starch (Atlas, 1993) and degradation of xylan and cellulose (Ten *et al.*, 2004) were also investigated; reactions were read after 5 days. Growth at different temperatures (4, 10, 18, 30, 37, 42 and 45 °C) and various pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed with R2A agar after 5 days of incubation. The following biological buffers (50 mM) were used to adjust the pH of the media: Na_2HPO_4/NaH_2PO_4 for pH 5.0–8.0 and $Na_2CO_3/NaHCO_3$ for pH 8.5–10.0 (Bates & Bower, 1956; Gomori, 1955). Salt tolerance was tested on R2A agar supplemented with 1–10 % (w/v) NaCl after 5 days incubation at 30 °C.

Extraction of genomic DNA was performed using a commercial genomic DNA extraction kit (Core Biosystem). PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified PCR product

were carried out using six primers, according to Kim *et al.* (2005), to almost cover the complete sequence of the 16S rRNA gene. The sequences were compiled using SeqMan software (DNASTAR) and resulted in a partial sequence of 1449 bp. The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Sequence similarity values were calculated by the EzTaxon server (Chun *et al.*, 2007). Multiple alignments were performed using CLUSTAL_X (Thompson *et al.*, 1997). Gaps were edited in the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983) and pairwise deletion. The phylogenetic tree was constructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods (Fitch, 1971) using MEGA4 software (Kumar *et al.*, 2007). The software package PHYLIP version 3.67 (Felsenstein, 2004) was used for the phylogenetic analysis based on the maximum-likelihood algorithm. The resultant tree topologies were evaluated using bootstrap analysis (Felsenstein, 1985) based on 1000 replications for the neighbour-joining and maximum-parsimony methods and on 100 resampled datasets for the maximum-likelihood algorithm.

To measure the G+C content of the chromosomal DNA, genomic DNA was extracted from strain Gsoil 346^T and purified as described by Moore & Dowhan (1995), enzymically degraded into nucleosides and analysed as described by Mesbah *et al.* (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under a vacuum and re-extracted in *n*-hexane/water (1:1, v/v), purified from the *n*-hexane fraction using Sep-Pak (Waters) and analysed by HPLC, as described by Hiraishi *et al.* (1996). Cellular fatty acids were analysed from bacterial cells grown on trypticase soy agar (TSA; Difco) for 2 days at 30 °C. The cellular fatty acids were converted to the corresponding methyl esters, extracted according to the protocol of the Sherlock Microbial Identification System (MIDI), analysed by gas chromatography (Hewlett Packard 6890) and identified by the Microbial Identification software package (Sasser, 1990). The presence of diaminopimelic acid isomers in the cell-wall peptidoglycan was determined by TLC after hydrolysis with 6 M HCl at 100 °C for 18 h as described by Komagata & Suzuki (1987). Polar lipids were extracted, separated and identified by two-dimensional TLC and staining with molybdenum blue spray (M1942; Sigma), as described by Minnikin *et al.* (1977).

Strain Gsoil 346^T was Gram-positive, strictly aerobic, non-endospore-forming, non-motile and short rod-shaped. Colonies grown on R2A agar plates at 30 °C for 4 days were smooth, circular, light yellowish white in colour, convex and 1–3 mm in diameter. The temperature range for growth of strain Gsoil 346^T on R2A agar was 10–42 °C (optimum 18–37 °C) and there was no growth at 4 °C or 45 °C. The physiological characteristics of strain Gsoil 346^T are summarized in the species description and a comparison of selective characteristics with those of related type strains is given in Table 1.

Table 1. Physiological characteristics of strain Gsoil 346^T and closely related type strains of species in the genus *Nocardioides*

Strains: 1, *Nocardioides panacisoli* sp. nov. Gsoil 346^T (data from this study); 2, *N. aromaticivorans* DSM 15131^T (Kubota *et al.*, 2005); 3, *N. nitrophenolicus* JCM 10703^T (Yoon *et al.*, 1999); 4, *N. simplex* JCM 1363^T (Suzuki & Komagata, 1983; Yoon *et al.*, 1999; Kubota *et al.*, 2005); 5, *N. kongjuensis* KCTC 19054^T (Yoon *et al.*, 2006). All strains were positive for catalase and alkaline phosphatase. All strains were negative for esterase lipase (C8), α - and β -galactosidases and α -mannosidase. +, Positive; w, weakly positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Cell shape	Rods	Rods	Rods	Irregular rods	Rods, cocci
Motility	–	–	+	+	–
Conditions for growth					
Temperature (°C)	10–42	22–40	15–40	ND	10–40
pH	5.5–8.5	5.0–8.0	6.0–10.0	ND	5.5–8.0
Oxidase	+	–	+	+	+
Urease	–	+	ND	–	–
Hydrolysis of:					
Casein	–	+	+	–	+
Gelatin	+	ND	ND	+	+
Starch	–	–	+	+	–
Carbon source utilization:					
L-Arabinose	–	+	–	–	–
Cellobiose	–	+	–	–	–
Citrate	+	–	–	–	–
D-Fructose	+	+	+	–	–
Glycerol	–	w	–	–	–
Maltose	+	+	–	–	–
D-Mannitol	–	+	–	–	ND
D-Mannose	–	w	w	–	w
L-Rhamnose	–	w	+	–	ND
D-Ribose	–	+	+	–	–
D-Xylose	–	+	+	–	–
API ZYM					
Acid phosphatase	+	–	+	w	+
Cystine arylamidase	–	–	w	w	–
Esterase (C4)	–	+	–	–	–
α -Glucosidase	+	ND	ND	+	ND
β -Glucosidase	–	–	w	w	–
Lipase (C14)	–	ND	ND	–	ND
Naphthol-AS-BI-phosphohydrolase	+	–	+	–	w
Trypsin	+	–	+	+	–
Valine arylamidase	–	–	+	w	–
Cell-wall peptidoglycan*	LL-DAP	LL-DAP	LL-DAP	LL-DAP, glycine	LL-DAP
DNA G + C content (mol%)	73.0	72.0–72.4	71.4	72.0–73.5	72.1

*DAP, diaminopimelic acid.

The 16S rRNA gene sequence of strain Gsoil 346^T determined in this study was a continuous stretch of 1449 bp. The closest relative of strain Gsoil 346^T was *Nocardioides aquiterrae* GW-9^T (96.6 % 16S rRNA gene sequence similarity), but strain Gsoil 346^T formed a monophyletic clade with *N. kongjuensis* A2-4^T, *N. aromaticivorans* H-1^T, *Nocardioides nitrophenolicus* NSP41^T and *Nocardioides simplex* ATCC 15799^T in a neighbour-joining phylogenetic tree, which was supported by a robust bootstrap value of 89 %. This tree topology was also supported by the maximum-likelihood algorithm (Fig. 1).

Strain Gsoil 346^T contained MK-8(H₄) as the major menaquinone and LL-2,6-diaminopimelic acid as the diagnostic cell-wall diamino acid. The DNA G + C content was 73.0 mol%. All of these chemotaxonomic characteristics supported the affiliation of strain Gsoil 346^T to the genus *Nocardioides*. The major fatty acid profile of strain Gsoil 346^T contained mainly iso-C_{16:0} (28.0 %), C_{18:1 ω 9c} (10.7 %) and C_{17:1 ω 8c} (9.0 %), which is similar to those of other *Nocardioides* species, but the minor fatty acid profile was significantly different from those of the most closely related species of the genus *Nocardioides*: the saturated fatty

Fig. 1. Neighbour-joining phylogenetic tree based on a comparative analysis of 16S rRNA gene sequences, showing the relationships between strain Gsoil 346^T and type strains of species in the genus *Nocardioideae*. Bootstrap values (>50%) based on 1000 replications are shown at branch nodes. Solid circles indicate that the corresponding branches were also recovered in maximum-parsimony and maximum-likelihood trees. Open circles indicate that the corresponding nodes were also recovered in the maximum-likelihood tree. Bar, 0.01 substitutions per nucleotide position.

acid C_{15:0} was unique to strain Gsoil 346^T, C_{16:0} 2-OH was found only in strain Gsoil 346^T and *N. kongjuensis* KCTC 19054^T, and C_{16:0} 10-methyl, found in *N. simplex* JCM 1363^T and *N. kongjuensis* KCTC 19054^T, was absent in strain Gsoil 346^T (see Supplementary Table S1, available in IJSEM Online). Phosphatidylinositol and phosphatidylglycerol were detected as the major polar lipids (see Supplementary Fig. S1).

On the basis of the 16S rRNA gene sequence analysis of strain Gsoil 346^T and the type strains of species of the genus *Nocardioideae* and unique phenotypic characteristics (Table 1 and Supplementary Table S1), we have demonstrated that strain Gsoil 346^T is not affiliated with any existing species of the genus *Nocardioideae*. Therefore, strain Gsoil 346^T should be placed in the genus *Nocardioideae* as a

representative of a novel species, for which we propose the name *Nocardioideae panacisoli* sp. nov.

Description of *Nocardioideae panacisoli* sp. nov.

Nocardioideae panacisoli (pa.na.ci.so'li. N.L. n. *Panax* -acis scientific name of ginseng; L. n. *solum* -i soil; N.L. gen. n. *panacisoli* of soil of a ginseng field, the source of isolation of the organism).

Cells are Gram-positive, strictly aerobic, non-spore-forming, non-motile and short rod-shaped, 0.2–0.4 µm in diameter and 0.8–1.2 µm in length after 3 days of growth at 30 °C on R2A agar. Colonies on R2A agar are smooth, circular, light yellowish white and convex after 4 days. Grow at 10–42 °C (optimum 18–37 °C), but not at 4 °C or 45 °C. Optimum pH for growth is pH 5.5–8.5. Growth

occurs in the absence of NaCl and in the presence of 2.0 % (w/v) NaCl, but not 3.0 % (w/v) NaCl. Catalase- and oxidase production are positive. Nitrate is reduced. Production of urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and β -galactosidase and Voges–Proskauer test are negative. Produces H₂S from sodium thiosulfate. Does not produce acid or gas from glucose, does not produce indole from tryptophan and does not degrade xylan, starch, chitin or cellulose. The following compounds are utilized as sole carbon sources: acetate, adipate, citrate, 3-hydroxybutyrate, gluconate, D-glucose, L-malate, maltose, L-proline, propionate, sucrose and valerate. The following compounds are not utilized as sole carbon sources: N-acetylglucosamine, L-alanine, L-arabinose, caprate, L-fucose, glycogen, L-histidine, 3-hydroxybenzoic acid, inositol, itaconate, 5-ketogluconate, L-lactic acid, malonate, D-mannitol, D-mannose, melibiose, phenylacetate, L-rhamnose, D-ribose, salicin, L-serine, D-sorbitol and suberate. According to the results from the API ZYM tests, 2-naphthyl phosphate (pH 8.5), 2-naphthyl phosphate (pH 5.4), N-benzoyl-DL-arginine 2-naphthylamide, N-glutaryl-phenylalanine 2-naphthylamide, naphthol-AS-BI-phosphate and 2-naphthyl α -D-glucopyranoside are hydrolysed, but 2-naphthyl butyrate, 2-naphthyl caprylate, 2-naphthyl myristate, L-leucyl 2-naphthylamide, L-valyl 2-naphthylamide, L-cystyl 2-naphthylamide, 6-bromo-2-naphthyl α -D-galactopyranoside, 2-naphthyl β -D-galactopyranoside, naphthol-AS-BI- β -D-glucuronide, 6-bromo-2-naphthyl β -D-glucopyranoside, 1-naphthyl N-acetyl- β -D-glucosaminide, 6-bromo-2-naphthyl α -D-mannopyranoside and 2-naphthyl α -L-fucopyranoside are not hydrolysed. MK-8(H₄) is the predominant menaquinone and iso-C_{16:0}, C_{18:1} ω 9c and C_{17:1} ω 8c are the major components of cellular fatty acids. The cell-wall peptidoglycan contains LL-2,6-diaminopimelic acid. The major polar lipids are phosphatidylinositol and phosphatidylglycerol. The genomic DNA G + C content of the type strain is 73.0 mol% (HPLC).

The type strain, Gsoil 346^T (=KCTC 19470^T=DSM 21348^T), was isolated from the soil of a ginseng field in Pocheon province, South Korea.

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