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Nocardioides ginsengisegetis sp. nov., Isolated from Soil of a Ginseng Field

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A Gram-positive, rod-shaped, non-spore-forming bacterium (Gsoil 485^T) was isolated from the soil of a ginseng field located in Pocheon province in South Korea. This bacterium was characterized in order to determine its taxonomic position by using the polyphasic approach. On the basis of 16S rRNA gene sequence similarity, strain Gsoil 485^T was shown to belong to the family *Nocardioidaceae* and related to *Nocardioides koreensis* (96.8% 16S rRNA gene sequence similarity), *Nocardioides basaltis* (96.7%), *Nocardioides salarius* (96.7%), and *Nocardioides sediminis* (96.5%). The sequence similarity with other species that had validly published names within the genus *Nocardioides* was less than 96.4%. Strain Gsoil 485^T was characterized chemotaxonomically as having LL-2,6-diaminopimelic acid in a cell-wall peptidoglycan, MK-8(H_4) as the predominant menaquinone, and iso-$C_{16:0}$, $C_{18:1}$ ω9*c* as the major fatty acids. The G+C content of genomic DNA was 71.6 mol%. The chemotaxonomic properties and phenotypic characteristics supported the affiliation of strain Gsoil 485^T to the genus *Nocardioides*. The results of both physiological and biochemical tests allowed for genotypic differentiation of strain Gsoil 485^T from the recognized *Nocardioides* species. Therefore, strain Gsoil 485^T is considered to represent the novel species, for which the name *Nocardioides ginsengisegetis* sp. nov. is proposed, with the type strain Gsoil 485^T (KACC 14269^T =KCTC 19469^T =DSM 21349^T).

***Keywords*:** 16S rRNA gene, polyphasic taxonomy, *N. ginsengisegetis*

The genus *Nocardioides* was first proposed by Prauser (1976). Currently, the genus is comprised of 47 recognized species, including the recently described species *Nocardioides basaltis* (Kim *et al*., 2009a), *Nocardioides caeni* (Yoon *et al*., 2009), *Nocardioides sediminis* (Dastager *et al.*, 2009), *Nocardioides ginsengisoli* (Cui *et al*., 2009), *Nocardioides humi* (Kim *et al*., 2009b), and *Nocardioides terrae* (Zhang *et al*., 2009).

While studying a cultivable aerobic bacterial community in the soil of a ginseng field located in Pocheon province in South Korea, a large number of novel bacterial strains were isolated. One of these isolates, designated strain Gsoil 485^T, appeared to be a member of the genus *Nocardioides* in the family *Nocardioidaceae* lineage and became the subject of a taxonomic investigation.

In the present study, we conducted a phylogenetic (16S rRNA gene), phenotypic, genotypic, and chemotaxonomic analyses to determine the precise taxonomic position of this strain. On the basis of the results obtained in this study, we propose that strain Gsoil 485^T should be placed in the genus *Nocardioides* as a type strain of the novel species, *Nocardioides ginsengisegetis* sp. nov.

Materials and Methods

Isolation of bacterial strain and culture condition

Strain Gsoil 485^T was originally isolated from soil of a ginseng field of Pocheon province in South Korea. This soil sample was well suspended with 50 mM phosphate buffer (pH 7.0) and spread on one fifth strength modified-R2A agar (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 acid, 0.25 g soytone, 0.5 g dextrose, 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 $CaCl_2$, agar 15 g, per 1 L) plates after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were incubated at 30°C for 1 month. Single colonies on the plates were purified by transferring them onto new plate either with modified R2A or one-half-strength modified-R2A agar and incubated again. One isolate, Gsoil 485^T, was cultured routinely on R2A agar (Difco) at 25°C and preserved in a glycerol solution (20%, w/v) at -70°C. The strain Gsoil 485^T was deposited to the Korean Collection for Type Cultures (=KCTC 19469^T) and German Collection of Microorganisms and Cell Cultures (=DSM 21349^T) and Korean Agricultural Culture Collection (=KACC 14269^T).

Phenotypic and biochemical characteristics

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology and motility was observed under a Nikon light microscope at ×1,000, with cells grown on R2A agar for 3 days at 25°C. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman (2002). Anaerobic growth was determined in serum bottles containing R2A broth supplemented with thioglycolate (1 g/L), and in which the upper air layer had been replaced with nitrogen. In addition, biochemical test were carried out using API 20NE, API ID 32GN, API 50 CH (suspended in AUX medium) and API ZYM test kits according to the instructions of the manufacturer (bioMérieux, France). Tests for degradation of DNA (using DNase agar from Scharlau, with DNase activity by flooding

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plates with 1 M HCl), casein, chitin, starch (Atlas, 1993), lipid (Kouker and Jaeger, 1987), xylan, and cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, and 42°C) and various pH values (pH 4.5-10.0 at intervals of 0.5 pH units) was assessed after 7 days of incubation. Salt tolerance was tested on R2A medium supplemented with 1-10% (w/v at intervals of 1% unit) NaCl after 7 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA), and MacConkey agar was also evaluated at 25°C.

PCR amplification, 16S rRNA gene sequencing, and phylogenetic analysis

For phylogenetic analysis of the isolated strains, genomic DNA was extracted using a commercial genomic DNA extraction kit (Solgent, Korea). The 16S rRNA gene was amplified from the chromosomal DNA of strain Gsoil 485^T by using a universal bacterial primer set, 9F [5′-GAGTTTGATCCTGGCTCAG-3′; positions 9-27 (*Escherichia coli* 16S rRNA numbering)] and 1512R [5′-ACGGTTACCTTGTTACGACTT-3′; positions 1512-1492] (Weisburg *et al*., 1991). The purified PCR products were sequenced in Solgent (Korea). The primers used for full length sequencing were 536R [5′-GTATTACCGCGGCTGCTG-3′; positions 536-519], 805R [5′-GACTACCAGGGTATCTAATC-3′; positions 805-784], 907F [5′-AAACTCAAAKGAATTGACGG-3′; positions 907-926], and 1512 R.

Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR, USA). The 16S rRNA gene sequences of related taxa were obtained from GenBank and EzTaxon server (Chun *et al.*, 2007). Multiple alignments were performed by 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). The phylogenetic trees were constructed by using the neighbor-joining (Saitou and Nei, 1987) and the maximum-parsimony (Fitch, 1971) methods with the MEGA4 Program (Kumar *et al.*, 2008) with bootstrap values based on 1,000 replications (Felsenstein, 1985).

Determination of DNA G+C content

For measurement of the G+C content of chromosomal DNA, the genomic DNA of strain Gsoil 485^T was extracted and purified as described by Moore and Dowhan (1995) and enzymatically degraded into nucleosides. The G+C content was then determined as described by Mesbah *et al.* (1989), using reverse-phase HPLC.

Isoprenoid quinones, cellular fatty acids, and DAP analysis

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). The crude quinone in *n*-hexane solution was purified using Sep-Pak Vac Cartridges Silica (Waters) and subsequently analyzed by HPLC, as described by Hiraishi *et al*. (1996). Cellular fatty acid profiles were determined for strains grown on R2A agar (Difco) for 48 h at 28°C. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analyzed by gas chromatography (model 6890; Hewlett Packard) using the Microbial Identification software package (Sasser, 1990). The value range was obtained by duplicate experiments. The presence of diaminopimelic acid (DAP) isomers in the cell wall peptidoglycan was determined by using thin-layer chromatography after hydrolysis with 6 N HCl at 100°C for 18 h described by Komagata and Suzuki (1987).

Results and Discussion

Morphological and phenotypic characteristics

Cells of strain Gsoil 485^T were Gram-positive, aerobic, non-spore-forming, non-motile, short-rod shaped, oxidase-positive, and catalase-positive. The colonies grown on R2A agar plates (Difco) for 3 days were smooth, circular, creamy-white in color, convex, and 1-1.5 mm in diameter. On R2A agar, Gsoil 485^T was able to grow at 4-37°C, but not 42°C. The isolate grew on nutrient agar and TSA (Difco), but not on MacConkey agar. The isolate hydrolyzed the gelatin, indicating proteolytic activity, but it did not hydrolyze the casein. The capability to degrade starch and Tween 80, which is present in closely related *Nocardioides* species (Dastager *et al.*, 2008; Kim *et al.*, 2008), was not observed in our strain. The phenotypic and chemotaxonomic characteristics that differentiate the strain Gsoil 485^T from other *Nocardioides* species are listed in Table 1.

Cellular fatty acid, quinone, and DAP composition

The respiratory quinone system supported the affiliation of strain Gsoil 485^T to the genus *Nocardioides*, where the majority of species of the genus *Nocardioides* had MK-8(H_4) as the predominant quinone. The presence of LL-diaminopimelic acid as the cell-wall peptidoglycan also supported the placement of the strain Gsoil 485^T in the genus *Nocardioides*. The cellular fatty acids of strain Gsoil 485^T and related strains are listed in Table 2. As shown in Table 2, although there were some differences between the cellular fatty acids of strain Gsoil 485^T and related strains, the predominant fatty acid of all the 5 compared strains was iso-$C_{16:0}$, which ranged from 26.9-70.3% of the total fatty acids. The most fatty acid of the strain Gsoil 485^T was iso-$C_{16:0}$ (26.9%) and $C_{18:1}$ ω9*c* (13.7%). In particular, the strain Gsoil 485^T differed from other *Nocardioides* species due to its higher content of $C_{16:0}$, $C_{18:1}$ ω9*c*, 10-methyl $C_{18:0}$, and absence of iso-$C_{18:0}$.

DNA G+C content

The DNA G+C content of strain Gsoil 485^T was 71.6 mol%, similar to those of *N. koreensis*, *N. basaltis*, *N. salaries*, and *N. marinisabuli*, which were in the range of 68.0-73.0 mol%.

Phylogenetic analysis

The 16S rRNA gene sequences of the strain Gsoil 485^T determined in this study were continuous stretches of 1,426 bp (base position 21-1470 with respect to the *Escherichia coli* numbering system), which were deposited in a GenBank database (accession numbers GQ339901). A sequence similarity calculation from using the EzTaxon server [http://www.eztaxon.org/; Chun *et al*. (2007)] indicated that the closest relatives of strain Gsoil 485^T were *N. koreensis* MSL-09^T (96.8%), *N. basaltis* J112^T (96.7%), *N. salaries* CL-Z59^T (96.7%), *N. sediminis* (96.5%), *N. terrigena* (96.4%), *N. marinisabuli* SBS-12^T (96.3%), and *N. dokdonensis* FR1436^T (96.1%). This relationship between strain Gsoil 485^T and other members of the genus *Nocardioides* was also evident in the phylogenetic tree, which used over 1,400 nt (Fig. 1). Strain Gsoil 485^T and *Nocardioides koreensis* MSL-09^T formed a monophyletic clade with a bootstrap high value of 99%, which was supported by both tree making methods used in this study. The generally

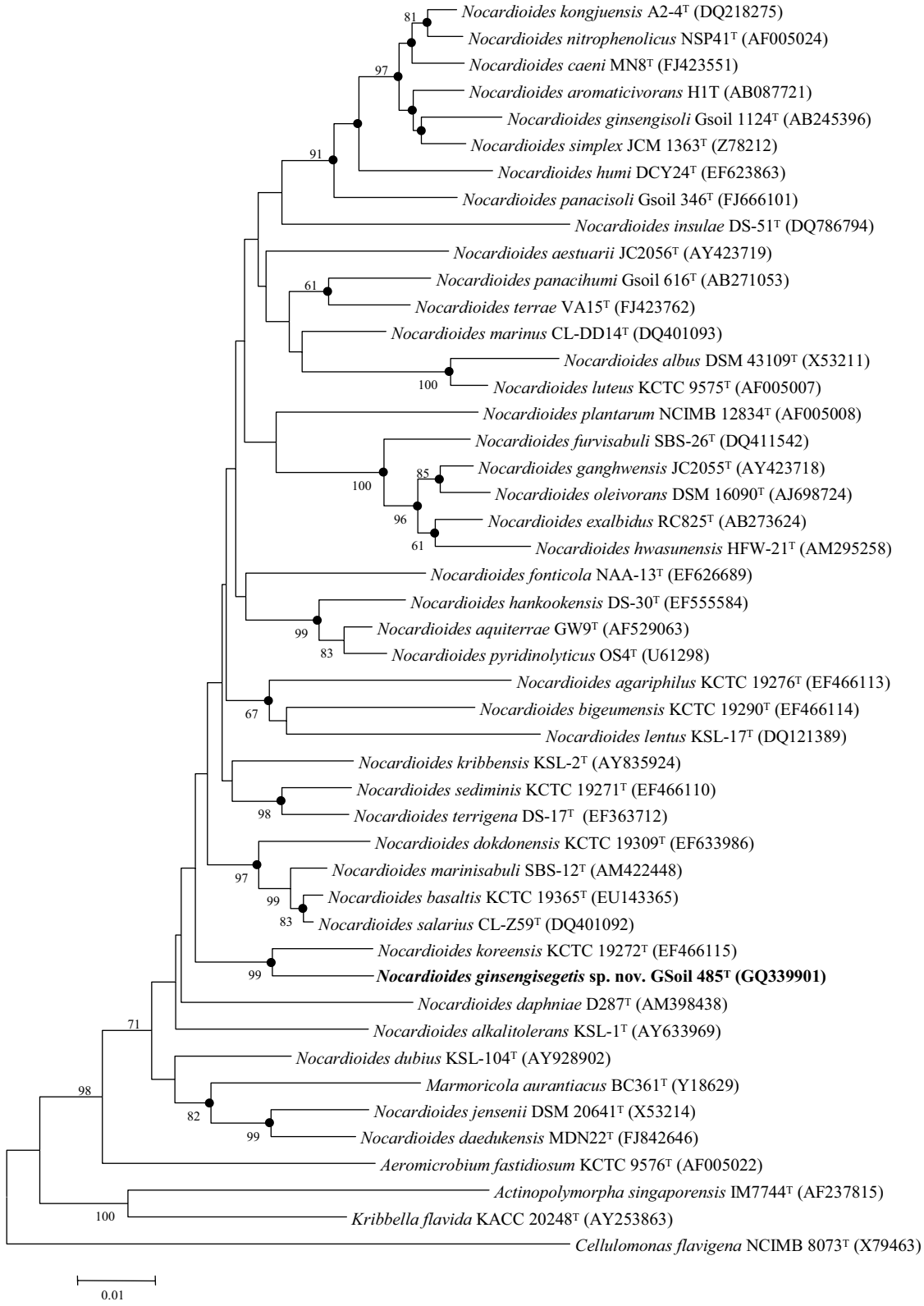

Fig. 1. Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of *Nocardioides ginsengisegetis* Gsoil 485^T with other related species. This tree was made using the neighbor-joining method (Saitou and Nei, 1987) with a Kimura (1983) two-parameter distance matrix and pairwise deletion. Dots indicate generic branches that were also recovered by using maximum-parsimony algorithms. Bootstrap values (expressed as percentages of 1,000 replications) greater than 60% are shown at the branch points. Bar, 0.01 substitutions per 1 nucleotide position.

Table 1. Physiological characteristics of *Nocardioides ginsengisegetis* Gsoil 485^T and related species belonging to the genus *Nocardioides*
Strains: 1, *Nocardioides ginsengisegetis* Gsoil 485^T; 2, *Nocardioides koreensis* MSL-09^T (data in column 1-2 from this study); 3, *Nocardioides marinisabuli* SBS-12^T (Lee *et al*., 2007); 4, *Nocardioides dokdonensis* FR1436^T (Park *et al*., 2008); 5, *Nocardioides basaltis* J112^T (Kim *et al*., 2009a); 6**,** *Nocardioides salaries* CL-Z59^T (Kim *et al*., 2008).
+, Positive; (+), weakly positive; -, negative; ND, no data available. All strains are negative for utilization of *myo*-inositol, raffinose, and L-rhamnose. All strains are positive for alkaline phosphatase, esterase lipase (C8). All strains are negative for *β*-glucuronidase and *α*-fucosidase.

Characteristics	1	2	3	4	5	6
Isolation source	Ginseng field soil	Soil	Beach sand	Beach sand	Beach sand	Zooplankton-enriched seawater
Cell morphology	Short rods	Short rods	Rods	Short rods	Short rods	Rods
Cell size (μm)	0.3-0.4 × 0.9-1.3	0.2-0.7 × 0.8-3.2	0.6-0.8 × 1.4-2.1	0.6-0.9 × 1.2-1.8	0.7-1.0 × 1.2-2.0	0.3-0.6 × 0.6-1.6
Motility	−	+	−	ND	−	-
Colony color	Cream-white	Cream-white	Pale yellow	Creamy	Creamy	Creamy
Temperature range (optimum) °C	4-37 (25-30)	27-37 (30)	4-40	4-30 (25)	10-37 (25-30)	10-35 (25-30)
pH range (optimum)	5.5-9.5	(7.0-8.0)	5.1-12.1 (7.1-8.1)	5-10 (7)	5.5-8.0 (6-7)	6-10 (6-7)
NaCl range (optimum) %	0-3	0-5	0-8	0-7 (0-3)	1-10 (1-2)	1-10 (3)
Oxidase	+	-	-	-	-	-
Nitrate reduction	+	-	-	+	-	-
Gelatinase	+	-	ND	−	+	+
Catalase	+	-	+	+	ND	+
Decomposition of						
Starch	-	+	w	-	-	w
Tween 80	-	+	-	+	-	+
Tyrosine	-	+	-	-	-	+
Aesculin	+	+	+	-	-	+
Casein	-	-	+	-	ND	+
Urea	-	-	-	+	ND	-
API ZYM test results						
Esterase (C4)	-	+	-	+	+	+
Lipase (C14)	-	-	-	-	-	-
Leucine arylamidase	+	+	+	+	+	+
Valine arylamidase	-	+	-	w	-	+
Cystine arylamidase	-	-	-	-	w	+
Trypsin	-	-	-	+	w	+
α-Chymotrypsin	-	-	-	+	-	+
Acid phosphatase	+	+	-	+	-	+
Naphtol-AS-BI-phosphohydrolase	+	+	-	+	+	+
α-Galactosidase	-	+	-	-	-	-
β-Galactosidase	+	-	-	-	-	-
α-Glucosidase	+	+	+	+	+	+
β-Glucosidase	+	+	-	-	-	-
α-Mannosidase	-	-	-	-	-	-
Carbon utilization						
N-Acetyl-D-glucosamine	-	-	+	-	-	-
L-Arabinose	-	-	-	-	-	-
Cellobiose	+	-	-	ND	+	+
Citrate	-	-	+	+	-	-
D-Fructose	-	-	+	+	-	-
D-Galactose	-	-	+	-	-	-
D-Glucose	+	+	−	−	+	+
Lactose	+	+	-	-	-	-
Maltose	-	-	+	-	-	ND
D-Mannitol	+	-	-	+	+	+
D-Mannose	-	-	-	+	-	-
D-Ribose	+	-	-	+	-	+
Salicin	-	-	-	-	+	-
Sucrose	-	+	-	-	+	+
Trehalose	+	-	ND	+	+	+
DNA G+C content (mol%)	71.6	69.9*	73	69.1	68	73

* Data was from Dastager *et al*. (2008)

Table 2. Cellular fatty acid profiles of strain Gsoil 485^T and recognized *Nocardioides* species
Strains: 1, *Nocardioides ginsengisegetis* Gsoil 485^T; 2, *Nocardioides koreensis* MSL-09^T; 3, *Nocardioides marinisabuli* SBS-12^T; 4, *Nocardioides dokdonensis* FR1436^T (data in columns 1-4 from this study, R2A at 28°C for 2 days); 5, *Nocardioides basaltis* J112^T (Marine agar at 30°C for 3 days data from Kim *et al*., 2009a); 6, *Nocardioides salaries* CL-Z59^T (Marine agar at 30°C for 1 day data from Kim *et al*., 2008).
All strain's fatty acids that account for less than 0.5% of the total fatty acids are not shown. Therefore, the percentages do not add up to 100%.

Fatty acid	1	2	3	4	5	6
Saturated fatty acid						
$C_{14:0}$	0.6	-	1.0	0.6	-	-
$C_{16:0}$	5.7	1.3	4.9	5.1	1.3	1.0
$C_{17:0}$	3.2	4.4	2.3	1.5	1.2	-
$C_{18:0}$	1.8	1.0	-	-	-	-
Unsaturated fatty acid						
$C_{15:1}$ $\omega 6c$	-	-	1.1	-	-	-
$C_{17:1}$ $\omega 6c$	-	-	0.9	-	-	-
$C_{17:1}$ $\omega 8c$	9.9	12.5	6.1	4.7	4.3	-
$C_{18:1}$ $\omega 9c$	13.7	8.6	2.1	3.3	2.8	1.4
Branched chain fatty acid						
iso-$C_{14:0}$	1.3	0.9	3.9	7.0	3.5	3.2
iso-$C_{15:0}$	7.4	3.9	3.3	3.0	-	3.4
iso-$C_{16:0}$	26.9	38.0	55.8	55.5	70.3	65.3
iso-$C_{16:1}$ H	1.9	3.7	0.8	1.6	3.7	4.6
iso-$C_{17:0}$	3.5	3.1	1.6	1.6	-	2.5
iso-$C_{18:0}$	-	0.8	0.7	0.9	2.7	1.4
anteiso-$C_{15:0}$	0.8	1.3	1.1	0.9	-	-
anteiso-$C_{17:0}$	1.6	6.7	3.1	2.6	-	2.3
anteiso-$C_{17:1}$ ω9c	-	0.9	-	-	-	-
Methyl ester fatty acid						
10-methyl $C_{16:0}$	2.8	3.2	-	-	-	5.2
10-methyl $C_{17:0}$	7.2	4.6	-	-	3.2	1.4
10-methyl $C_{18:0}$ (TBSA)	5.6	1.2	-	-	-	-
Summed feature[a]						
3	3.4	2.0	9.4	9.6	-	-
6	-	1.2				
8	1.9	0.9	2.0	2.2	1.7	2.4

[a] Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed features consist of: 3, $C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$; 6, $C_{19:1}$ $\omega 11c$/$C_{19:1}$ $\omega 9c$; 8, $C_{18:1}$ $\omega 7c$/$C_{18:1}$ $\omega 6c$.

recommended and accepted criteria for delineating bacterial species have stated that strains with a DNA-DNA relatedness below 70%, as measured by hybridization, or with a 16S rRNA gene sequence dissimilarity above 3%, are considered to be a separate species (Stackebrandt and Goebel, 1994). Taking into account this definition, the above mentioned data indicate that strain Gsoil 485^T has a high enough probability to be a novel species of the genus *Nocardioides.*

Taxonomic conclusions

The phenotypic and phylogenetic characterizations indicated that strain Gsoil 485^T belongs to the genus *Nocardioides*. There were some phenotypic differences between the strain Gsoil 485^T and the phylogenetically related *Nocardioides* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 485^T should be classified within the genus *Nocardioides* as the type strain of novel species, for which the name *Nocardioides ginsengisegetis* sp. nov. is proposed.

Description of *Nocardioides ginsengisegetis* sp. nov.

Nocardioides ginsengisegetis (*gin.seng.i.se.ge.ti's*.. N.L. n. *ginsengum*, ginseng; *seges segetis*, a field; N.L. gen. n. *ginsengisegetis*, of a ginseng field)

Cells are Gram-positive, aerobic, non-spore-forming, non-motile, and short-rod shaped, 0.3-0.4 μm in diameter and 0.9-1.3 μm in length after culture on R2A agar for 3 days. Colonies grown on R2A agar (Difco) for 3 days are smooth, circular, translucent, creamy-white, and convex. Grows at 4-37°C and in pH 5.5-9.5, but not at 42°C. Optimum growth occurs at 25-30°C and pH 7.0. Growth occurs in the absence of NaCl and in the presence of 3.0% (w/v) NaCl, but not 4.0% (w/v) NaCl. Growth occurs on nutrient agar but not on MacConkey agar. Catalase-positive and oxidase-positive. Nitrate are reduced in aerobic conditions. Urease, arginine dihydrolase, indole production are negative. β-Galactosidase, β-glucosidase, and gelatin hydrolysis are positive. Does not produce any acid or gas from glucose. Does not degrade starch,

cellulose, DNA, xylan, chitin, xanthine, and hypoxanthine. Carbon assimilation tests as a sole carbon sources (API ID 32 GN & API 50CH) and enzyme activities (API ZYM) are listed in Table 1. In addition, the following compounds are utilized as sole carbon sources: D-arabinose, acetate, L-proline, 3-hydroxybutyrate, valerate, and propionate. The following compounds are not utilized as sole carbon sources: 2-ketogluconate, 3-hydroxy-benzoate, 4-hydroxy-benzoate, 5-ketogluconate, 5-ketogluconate, adonitol, amidon, amygdalin, arbutin, caprate, citrate, D-arabitol, D-lyxose, D-maltose, D-mannose, D-melezitose, D-melobiose, D-raffinose, D-sorbitol, D-tagatose, D-turanose, dulcitol, erythritol, gentiobiose, gluconate, glycogen, inositol, inulin, itaconate, lactate, L-alanine, L-arabitol, L-fucose, L-histidine, L-rhamnose, L-serine, L-sorbose, L-xylose, malonate, and xylitol. MK-8(H_4) is the predominant menaquinone, and iso-$C_{16:0}$, $C_{18:1}$ $\omega 9c$ are the major components of cellular fatty acids. The G+C content of genomic DNA is 71.6 mol%. The cell wall peptidoglycan of strain Gsoil 485^T contains LL-DAP.

The type strain, Gsoil 485^T (=KACC 14269^T =KCTC 12600^T =DSM 21439^T) was isolated from soil of a ginseng field of Pocheon Province, South Korea.

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References

Atlas, R.M. 1993. Handbook of Microbiological Media. CRC Press, Boca Raton, Florida, USA.

Buck, J.D. 1982. Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl. Environ. Microbiol.* 44, 992-993.

Cappuccino, J.G. and N. Sherman. 2002. Microbiology: a laboratory manual, 6th ed. Pearson Education, Inc., California, USA.

Chun, J., J.H. Lee, Y. Jung, M. Kim, S. Kim, B.K. Kim, and Y.W. Lim. 2007. EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int. J. Syst. Evol. Microbiol.* 57, 2259-2261.

Cui, Y.S., S.T. Lee, and W.T. Im. 2009. *Nocardioides ginsengisoli* sp. nov., isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol*. 59, 3045-3050.

Dastager, S.G., J.C. Lee, Y.J. Ju, D.J. Park, and C.J. Kim. 2008. *Nocardioides koreensis* sp. nov., *Nocardioides bigeumensis* sp. nov. and *Nocardioides agariphilus* sp. nov., isolated from soil from Bigeum Island, Korea. *Int. J. Syst. Evol. Microbiol.* 58, 2292-2296.

Dastager, S.G., J.C. Lee, Y.J. Ju, D.J. Park, and C.J. Kim. 2009. *Nocardioides sediminis* sp. nov., isolated from a sediment sample. *Int. J. Syst. Evol. Microbiol*. 59, 280-284.

Felsenstein, J. 1985. Confidence limit on phylogenies: an approach using the bootstrap. *Evolution* 39, 783-791.

Fitch, W.M. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Zool.* 20, 406-416.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95-98.

Hiraishi, A., Y. Ueda, J. Ishihara, and T. Mori. 1996. Comparative lipoquinone analysis of influent sewage and activated sludge by high-performance liquid chromatography and photodiode array detection. *J. Gen. Appl. Microbiol.* 42, 457-469.

Kim, H.M., D.H. Choi, C.Y. Hwang, and B.C. Cho. 2008. *Nocardioides salarius* sp. nov., isolated from seawater enriched with zooplankton. *Int. J. Syst. Evol. Microbiol.* 58, 2056-2064.

Kim, K.H., S.W. Roh, H.W. Chang, Y.D. Nam, J.H. Yoon, C.O. Jeon, H.M. Oh, and J.W. Bae. 2009a. *Nocardioides basaltis* sp. nov., isolated from black beach sand. *Int. J. Syst. Evol. Microbiol*. 59, 42-47.

Kim, M.K., S. Srinivasan, M.J. Park, G. Sathiyaraj, Y.J. Kim, and D.C. Yang. 2009b. *Nocardioides humi* sp. nov., a β-glucosidase-producing bacterium isolated from soil of a ginseng field. *Int. J. Syst. Evol. Microbiol*. 59, 2724-2728.

Kimura, M. 1983. The Neutral Theory of Molecular Evolution. Cambridge: Cambridge University Press, Cambridge, New York, N.Y., USA.

Komagata, K. and K. Suzuki. 1987. Lipids and cell-wall analysis in bacterial systematics. *Methods Microbiol.* 19, 161-203.

Kouker, G. and K.E. Jaeger. 1987. Specific and sensitive plate assay for bacterial lipases. *Appl. Environ. Microbiol.* 53, 211-213.

Kumar, S., J. Dudley, M. Nei, and K. Tamura. 2008. MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief. Bioinform.* 9, 299-306.

Lee, D.W., C.G. Hyun, and S.D. Lee. 2007. *Nocardioides marinisabuli* sp. nov., a novel actinobacterium isolated from beach sand. *Int. J. Syst. Evol. Microbiol*. 57, 2960-2963.

Mesbah, M., U. Premachandran, and W. Whitman. 1989. Precise measurement of the G+C content of deoxyribonucleic acid by high performance liquid chromatography. *Int. J. Syst. Bacteriol.* 39, 159-167.

Moore, D.D. and D. Dowhan. 1995. Preparation and analysis of DNA, pp. 2-11. *In* F.W. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl (eds.), Current Protocols in Molecular Biology. Wiley, New York, N.Y., USA.

Park, S.C., K.S. Baik, M.S. Kim, J. Chun, and C.N. Seong. 2008. *Nocardioides dokdonensis* sp. nov., an actinomycete isolated from sand sediment. *Int. J. Syst. Evol. Microbiol.* 58, 2619-2623.

Prauser, H. 1976. *Nocardioides*, a new genus of the order *Actinomycetales*. *Int. J. Syst. Bacteriol*. 26, 58-65.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol*. 4, 406-425.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, DE, USA.

Stackebrandt, E. and B.M. Goebel. 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* 44, 846-849.

Ten, L.N., W.T. Im, M.K. Kim, M.S. Kang, and S.T. Lee. 2004. Development of a plate technique for screening of polysaccharide-degrading microorganisms by using a mixture of insoluble chromogenic substrates. *J. Microbiol. Methods* 56, 375-382.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876-4882.

Weisburg, W.G., S.M. Barns, D.A. Pelletier, and D.J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* 173, 697-703.

Yoon, J.H., S.J. Kang, S. Park, W. Kim, and T.K. Oh. 2009. *Nocardioides caeni* sp. nov., isolated from wastewater. *Int. J. Syst. Evol. Microbiol*. 59, 2794-2797.

Zhang, J.Y., X.Y. Liu, and S.J. Liu. 2009. *Nocardioides terrae* sp. nov., isolated from forest soil. *Int. J. Syst. Evol. Microbiol*. 59, 2444-2448.