Aeromicrobium ginsengisoli sp. nov., isolated from a ginseng field

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Strain Gsoil 098^T, a Gram-positive, non-spore-forming, non-motile coccus, was isolated from soil from a ginseng field in South Korea and characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain Gsoil 098^T belongs to the family *Nocardioidaceae*, and the highest degrees of sequence similarity were found with *Aeromicrobium marinum* T2^T (99.0%), *A. panaciterrae* Gsoil 161^T (98.9%), *A. alkaliterrae* KSL-107^T (98.4%), *A. fastidiosum* KCTC 9576^T (98.1%) and *A. erythreum* NRRL B-3381^T (97.5%). Chemotaxonomic analysis revealed that strain Gsoil 098^T possesses menaquinone MK-9(H₄) and predominant fatty acids C_{16:0}, 10-methyl C_{18:0} and C_{18:0}. DNA–DNA hybridization results and physiological and biochemical tests clearly demonstrated that strain Gsoil 098^T represents a distinct species. Based on these data, Gsoil 098^T (=KCTC 19207^T =JCM 14732^T =GBS 39^T) should be classified as the type strain of a novel *Aeromicrobium* species, for which the name *Aeromicrobium ginsengisoli* sp. nov. is proposed.

The genus *Aeromicrobium* belonging to the family *Nocardioidaceae* (Nesterenko *et al.*, 1985; Stackebrandt *et al.*, 1997) was established by Miller *et al.* (1991) with a single species, *Aeromicrobium erythreum*. Subsequently, *Nocardioides fastidiosa* was transferred to the genus as *Aeromicrobium fastidiosum* (Tamura & Yokota, 1994). Four further species have been described: *Aeromicrobium marinum* (Bruns *et al.*, 2003), *A. alkaliterrae* (Yoon *et al.*, 2005), *A. tamulense* (Lee & Kim, 2007) and *A. panaciterrae* (Cui *et al.*, 2007).

In a series of studies, we have attempted to isolate micro-organisms from a ginseng field in order to investigate the community structure based on a culture-dependent method. In this study, a strain was isolated from the soil of a ginseng field in Daejeon (South Korea) and characterized using a polyphasic approach, including phylogenetic analyses based on 16S rRNA gene sequences, genomic relatedness and chemotaxonomic and phenotypic properties in order to determine the precise taxonomic position of strain Gsoil 098^T. The results indicate that Gsoil 098^T is a member of the genus *Aeromicrobium*, but it is clearly distinguished from the current *Aeromicrobium* species with validly published names.

Strain Gsoil 098^T was isolated from soil via direct plating onto R2A agar (Difco). Single colonies on these plates were purified by transferring them onto new plates four times

and subjecting them to additional incubation for 5 days at 30 °C. Purified colonies were tentatively identified using partial 16S rRNA gene sequences. Strain Gsoil 098^T was deposited in the Ginseng Genetic Resource Bank (<http://www.ginsengbank.org>) as strain GBS 39^T.

Cell morphology and motility were observed with a Nikon light microscope (×1000 magnification) with cells grown for 3 days at 30 °C on R2A agar. The Gram reaction was determined according to the non-staining method described by Buck (1982). Oxidase activity was evaluated via oxidation of 1% *p*-aminodimethylaniline oxalate and catalase activity was determined by measuring bubble production after application of a 3% (v/v) hydrogen peroxide solution. Acid production from carbohydrates was assessed using the procedures outlined by Cappuccino & Sherman (2002). Growth at various temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar and growth at different pH values (pH 5.0–10.0 at intervals of 0.5 pH units) was assessed in R2A broth. Salt tolerance was tested on R2A agar supplemented with 1–10% (w/v) NaCl after 5 days incubation. Growth was also evaluated at 30 °C on nutrient agar, trypticase soy agar (TSA) and MacConkey agar. The API 20E, API 20NE and API ID32 GN microtest systems were employed in these tests according to the recommendations of the manufacturer (bioMérieux). Nitrate reduction was tested using API 20NE (bioMérieux).

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

Abbreviation: TBSA, tuberculostearic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 098^T is AB245394.

Table 1. Differential phenotypic characteristics between strain Gsoil 098^T and type strains of *Aeromicrobium* species

Strains: 1, strain Gsoil 098^T; 2, *A. alkaliterrae* DSM 16824^T (unless indicated, data were taken from Yoon *et al.*, 2005); 3, *A. erythreum* DSM 8599^T (Park *et al.*, 1999); 4, *A. fastidiosum* DSM 10552^T (Park *et al.*, 1999); 5, *A. marinum* DSM 15272^T (Bruns *et al.*, 2003); 6, *A. panaciterrae* DSM 17939^T (Cui *et al.*, 2007); 7, *A. tamlense* JCM 13811^T (data taken from Lee & Kim, 2007). +, Positive; –, negative; w, weakly positive; ND, no data available. All strains were positive for production of β -glucosidase and assimilated D-galactose, D-glucose, trehalose and glycerol.

Characteristic	1	2	3	4	5	6	7
Motility	–	–	–	+	–	–	–
Morphology*	C	R, C	IR, C	R, C	R	R	IR
Cell size (μ m)							
Length	0.5–1.2	0.8–1.4	0.5–1.2	ND	0.7–1.3	1.0–1.5	0.8–4.8
Width	0.3–0.4	0.3–0.5	0.5	ND	0.3–0.5	0.2–0.4	0.4–0.6
Colony colour†	WH	CR	B to AB	ND	I	LYW	Y
Oxidase	+	–	+	+	–	–	–
Catalase	–	+	+	+	+	+	+
Optimum growth temperature (°C)	30	25	35	25	25	ND	30
Growth at/in:							
4 °C	+	+	–	ND	+	–	–
37 °C	–	–	+	ND	ND	–	+
42 °C	–	–	–	–	–	–	+
pH 5.5	+	–	+	+	+	+	+
4 % NaCl	w	+	+	+	+	–	+
Nitrate reduction‡	–	+	–	+	+	+	–
Activity of:							
Arginine dihydrolase‡	–	–	ND	–	–	–	–
Cellulase	–	–	+	–	–	–	ND
α -Glucosidase	–	+	+	+	–	–	+
β -Galactosidase‡	+	+	–	+	+	+	–
Lysine decarboxylase	–	–	ND	ND	ND	–	–
Ornithine decarboxylase	–	–	ND	ND	ND	–	–
Protease (gelatin hydrolysis)‡	+	+	+	–	–	+	ND
Tryptophan deaminase	–	–	ND	ND	ND	–	–
Urease‡	–	–	–	–	–	–	–
Assimilation of:‡							
3-Hydroxybutyrate	+	+	ND	+	+	–	ND
Acetate	+	+	+	+	+	–	+
Adipate	–	+	ND	+	+	+	ND
Caprate	–	+	ND	+	+	+	ND
Citrate	–	+	ND	+	+	+	–
Gluconate	+	+	ND	+	+	+	ND
DL-Lactate	–	–	ND	+	–	–	ND
Malate	+	+	ND	+	+	+	+
Malonate	–	+	ND	–	–	–	ND
Phenyl acetate	–	+	ND	+	+	+	ND
Propionate	+	+	ND	+	–	–	ND
Pyruvate	+	–	ND	+	+	ND	ND
Suberate	–	+	ND	+	–	–	ND
Succinate	+	+	+	–	+	+	+
Valerate	+	+	ND	+	–	–	ND
L-Arabinose	+	–	+	+	–	–	–
Cellobiose	+	+	–	+	+	+	+
D-Fructose	+	–	+	+	–	–	+
Maltose	+	+	ND	+	+	+	ND
D-Mannose	+	–	–	+	+	+	+
Rhamnose	+	+	ND	+	–	–	ND
Sucrose	+	+	+	+	–	–	+
Trehalose	+	+	+	ND	+	ND	+
D-Xylose	+	–	+	+	–	–	–

Table 1. cont.

Characteristic	1	2	3	4	5	6	7
Inositol	+	+	+	+	—	—	—
D-Mannitol	—	w	—	+	+	+	—
N-Acetyl-D-glucosamine	+	w	ND	—	w	w	ND
Salicin	—	+	—	—	—	+	—

*C, Cocci; IR, irregular rods, R, rods.

†AB, Amber-beige; B, beige; CR, cream; I, ivory; LYW, light yellowish white; WH, white; Y, yellow.

‡Determined in this study using API kits for all strains except *A. tamulense* JCM 13811^T.

acid methyl ester analysis, the strain was allowed to grow on TSA for 48 h at 30 °C; two loops of well-grown cells were then harvested. Fatty acid methyl esters were prepared, separated and identified using the Sherlock Microbial Identification System (MIDI, Inc.) (Sasser, 1990).

Genomic DNA for DNA–DNA hybridization was extracted and purified using the Qiagen Genomic-tip system 100/G and then enzymically degraded into nucleosides as described previously (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA–DNA hybridization was performed fluorometrically according to the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. The hybridization was conducted in five replications for each sample. The highest and lowest values obtained for each sample were excluded; DNA relatedness values quoted are means of the remaining three values.

Genomic DNA for sequencing was extracted and purified with the Genomic DNA isolation kit (Core Bio System). The 16S rRNA gene was amplified from chromosomal DNA of strain Gsoil 098^T using universal bacterial primers 9F and 1512R (Weisburg *et al.*, 1991); purified PCR products were sequenced by Genotec (Daejeon, South Korea) as noted in Kim *et al.* (2005). The full sequence of the 16S rRNA gene was compiled using SeqMan software and edited using BioEdit (Hall, 1999). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed using CLUSTAL_X (Thompson *et al.*, 1997), and evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1983). The phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) in MEGA 2 (Kumar *et al.*, 2001). A bootstrap analysis with 1000 replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). All species of the genus *Aeromicrobium* were included in the phylogenetic tree.

Strain Gsoil 098^T was cultured on R2A agar (Difco) at 30 °C and yielded white-coloured, circular colonies. Cells were aerobic, Gram-positive, non-motile cocci. The strain was able to grow at 4–30 °C, but not at 37 °C. Growth was not observed at 30 °C on MacConkey agar. Physiological characteristics of strain Gsoil 098^T are summarized in the

species description, and a comparison of selected characteristics with related type strains is shown in Table 1.

The cellular fatty acid profiles of strain Gsoil 098^T and related *Aeromicrobium* type strains are shown in Table 2. The major cellular fatty acids of strain Gsoil 098^T included C_{16:0} (32.7%), 10-methyl C_{18:0} (tuberculostearic acid; TBSA) (20.6%) and C_{18:0} (12.9%). Minor amounts of saturated fatty acids C_{17:0} (3.2%), 10-methyl C_{17:0} (2.4%) and 10-methyl C_{19:0} (3.1%) were also present. No significant differences in fatty acid profiles were found between strain Gsoil 098^T and *Aeromicrobium* species (Park *et al.*, 1999; Bruns *et al.*, 2003; Yoon *et al.*, 2005). TBSA and C_{16:0} are dominant fatty acids in *Aeromicrobium* species, including strain Gsoil 098^T, but are minor components in *Nocardioide*s species (Park *et al.*, 1999; Bruns *et al.*, 2003; Yi & Chun, 2004; Yoon *et al.*, 2004, 2005). The isolate contained a tetrahydrogenated menaquinone with nine isoprene units [MK-9(H₄)] as the predominant isoprenoid quinone. The menaquinone type differed from that found in the genera *Nocardioide*s, *Marmoricola* and *Actinopolymorpha* (Park *et al.*, 1999; Urzì *et al.*, 2000; Wang *et al.*, 2001; Yoon *et al.*, 2005).

The 16S rRNA gene sequence of strain Gsoil 098^T was a continuous stretch of 1483 nucleotides. Strain Gsoil 098^T was determined to belong to the subclass *Actinobacteridae*, order *Actinomycetales*, suborder *Propionibacterineae*, family *Nocardioideaceae*. The highest degrees of sequence similarity were found with *A. marinum* T2^T (99.0%), *A. panaciterrae* Gsoil 161^T (98.9%), *A. alkaliterrae* KSL-107^T (98.4%), *A. fastidiosum* KCTC 9576^T (98.1%) and *A. erythreum* NRRL B-3381^T (97.5%). The most closely related *Nocardioide*s strain, *Nocardioide*s *dubius* KCTC 9992^T, showed a lower degree of sequence similarity (94.8%). As seen in the phylogenetic tree (Fig. 1), strain Gsoil 098^T clearly belongs to the *Aeromicrobium* lineage, as verified by the high bootstrap values.

The G+C content of the genomic DNA of strain Gsoil 098^T was 66.8 mol%. Strain Gsoil 098^T exhibited relatively low levels of DNA–DNA relatedness to *A. alkaliterrae* DSM 16824^T (51.2%), *A. marinum* DSM 15272^T (22.5%) and *A. panaciterrae* DSM 17939^T (41.0%). These values are considerably below 70% (Wayne *et al.*, 1987), the

Table 2. Cellular fatty acid profiles of strain Gsoil 098^T and type strains of *Aeromicrobium* species

Strains: 1, strain Gsoil 098^T; 2, *A. alkaliterrae* DSM 16824^T (data from Yoon *et al.*, 2005); 3, *A. erythreum* DSM 8599^T (Park *et al.*, 1999); 4, *A. fastidiosum* DSM 10552^T (Park *et al.*, 1999); 5, *A. marinum* DSM 15272^T (Bruns *et al.*, 2003); 6, *A. panaciterrae* DSM 17939^T (Cui *et al.*, 2007); 7, *A. tamlense* JCM 13811^T (Lee & Kim, 2007). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. Major components in strain Gsoil 098^T are highlighted in bold.

Fatty acid	1	2	3	4	5	6	7
Saturated							
C _{10:0}	2.16						
C _{12:0}							1.40
C _{14:0}		1.00	0.70	0.60	0.70		1.00
C _{15:0}		3.20	1.70	1.00	1.40	4.90	1.00
anteiso-C _{15:0}							0.40
C _{16:0}	32.73	34.10	20.00	10.10	15.00	13.00	13.80
10-Methyl C _{16:0}		4.00				11.70	
C _{16:0} iso		0.80	1.40		1.60	1.50	
C _{17:0}	3.20	3.50	2.40	2.10	4.70	4.80	1.80
10-Methyl C _{17:0}	2.41	5.00	0.30			11.90	
C _{18:0}	12.94	1.20	6.10	9.70	3.30		13.60
10-Methyl C _{18:0} (TBSA)	20.57	16.30	24.20	32.90	12.50	14.50	8.70
iso-C _{18:0}			0.50		1.80		
10-Methyl C _{19:0}	3.11						
Unsaturated							
C _{16:1} ω 9 <i>c</i>			0.80	1.40	1.00		1.60
iso-C _{17:1} ω 9 <i>c</i>			0.40	0.70			
C _{17:1} ω 6 <i>c</i>			0.60			1.40	
C _{17:1} ω 8 <i>c</i>		2.40	0.80		1.40	4.70	
C _{17:1} ω 9 <i>c</i>				0.90			
C _{18:1} ω 9 <i>c</i>	9.75	5.70	25.60	25.10	28.50	5.30	47.20
Hydroxy							
C _{14:0} 2-OH					0.20		
C _{15:0} 2-OH			0.60	0.60	0.60	1.80	
C _{15:0} 3-OH	1.99						
C _{16:0} 2-OH	6.34	17.60	11.30	8.90	14.00	12.80	6.80
iso-C _{16:0} 2-OH					0.80		
C _{17:0} 2-OH			0.90	1.50	3.90	2.70	1.10
Summed features*							
C _{14:1} ω 9 <i>t</i> /C _{14:1} ω 9 <i>c</i>							0.70
C _{16:1} ω 7 <i>c</i> /iso-C _{15:0} 2-OH	3.33	3.40		0.90		6.60	

*Summed features contain fatty acids that could not be separated by GLC using the Microbial Identification System (MIDI).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain Gsoil 098^T and all *Aeromicrobium* species. The neighbour-joining method was used. Bootstrap values are expressed as percentages of 1000 replications at branch points. Bar, 0.01 substitutions per nucleotide position.

threshold that is generally regarded as delineating a genomic species. Thus, our results support the placement of strain Gsoil 098^T within a separate and previously unrecognized species, for which the name *Aeromicrobium ginsengisoli* sp. nov. is proposed.

Description of *Aeromicrobium ginsengisoli* sp. nov.

Aeromicrobium ginsengisoli (gin.sen.gi.so'li. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. *ginsengisoli* from ginseng soil).

Gram-positive, aerobic, non-motile cocci, 0.5–1.2 × 0.3–0.4 µm after growth on R2A agar (Difco) at 30 °C for

5 days. Colonies on R2A agar after 5 days are white and circular. The optimal growth temperature and pH are 30 °C and pH 7, with a growth range of pH 5.0–8.5. Grows in a salt concentration of 3 %, but growth is weak above 4 %. Reduces nitrate to nitrite, but not to nitrogen gas. The test for oxidase activity is positive and the test for catalase activity is negative. Produces β -glucosidase, protease and β -galactosidase, but not arginine dihydrolase, cellulase, chitinase, DNase, α -glucosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease or xylanase. Assimilates 3-hydroxybutyrate, acetate, fumarate, gluconate, malate, propionate, pyruvate, succinate, n-valerate, D-arabinose, L-arabinose, cellobiose, D-fructose, D-fucose, D-galactose, D-glucose, D-lactose, D-lyxose, maltose, D-mannose, L-rhamnose, L-sorbose, sucrose, trehalose, D-xylose, dulcitol, ethanol, glycerol, inositol, L-alanine, L-asparagine, glutamate, glutamine, L-leucine, L-phenylalanine, L-proline, L-serine, L-tryptophan, L-tyrosine, N-acetyl-D-glucosamine and inulin. Does not assimilate 2-ketogluconate, 3-hydroxybenzoate, 4-hydroxybenzoate, 5-ketogluconate, adipate, benzoate, caprate, citrate, formate, glutarate, itaconate, lactate, maleic acid, malonate, oxalate, phenyl acetate, suberate, tartrate, L-fucose, melibiose, raffinose, D-ribose, L-xylose, D-adonitol, myo-inositol, D-mannitol, methanol, D-sorbitol, xylitol, L-arginine, L-aspartate, L-cysteine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-methionine, L-threonine, L-valine, salicin, glycogen or dextran. The DNA G+C content of the type strain is 66.8 mol% as determined by HPLC. The predominant quinone is MK-9(H₄). Major cellular fatty acids include C_{16:0} (32.7 %), 10-methyl C_{18:0} (20.6 %) and C_{18:0} (12.9 %), while minor saturated fatty acids C_{17:0} (3.2 %), 10-methyl C_{17:0} (2.4 %) and 10-methyl C_{19:0} (3.1 %) are also present.

The type strain, Gsoil 098^T (=KCTC 19207^T=JCM 14732^T=GBS 39^T), was isolated from soil of a ginseng field in Daejeon, South Korea.

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