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Aeromicrobium panacisoli sp. nov. Isolated from Soil of Ginseng Cultivating Field

Muhammad Zubair Siddiqi[1,2] · Soon Youl Lee[1,2] · Kang Duk Choi[1,2] · Wan-Taek Im[1,2]

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Abstract
A Gram-positive, rod-shaped, non-spore-forming, and aerobic bacterium (Gsoil 137^T) was isolated from soil of a ginseng field of Pocheon province in South Korea and subjected to a polyphasic approach in order to determine its taxonomic position. On the basis of 16S rRNA gene sequence similarity, strain Gsoil 137^T was shown to belong to the family *Nocardioidaceae* and was closely related to *Aeromicrobium ginsengisoli* Gsoil 098^T (96.7%), *Aeromicrobium panaciterrae* (96.7%), and *Aeromicrobium halocynthiae* JCM 15749^T (96.6%). Being phylogenetic, it was most closely related to *Aeromicrobium halocynthiae* JCM 15749^T. The G+C content of the genomic DNA was 70.3 mol%. The diagnostic diamino acid of the cell wall peptidoglycan was LL-diaminopimelic acid. The predominant menaquinone was menaquinone MK-8 (H_4) and MK-7 (H4) was a minor compound. The major cellular fatty acids were $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ $\omega 9c$ and summed feature 4 ($C_{16:1}$ $\omega 7c$/$C_{15:0}$ iso 2-OH). All these data supported the affiliation of strain Gsoil 137^T to the genus *Aeromicrobium*. The results of physiological and biochemical tests enabled strain Gsoil 137^T to be differentiated genotypically and phenotypically from currently known *Aeromicrobium* species. Therefore, strain Gsoil 137^T represents a novel species of the genus *Aeromicrobium*, for which the name *Aeromicrobium panacisoli* sp. nov. is proposed. The type strain is Gsoil 137^T (= KCTC 19130^T = DSM 17940^T = CCUG 52475^T).

Introduction

The genus *Aeromicrobium* was first proposed by Miller in 1991 [15]. According to the original, the genus comprised non-mycelial, non-sporulating actinomycetes that produced the macrolide antibiotic erythromycin; the type strain is *Aeromicrobium erythreum*. In 1994, *Nocardioides fastidiosa* was transferred to the genus as *Aeromicrobium fastidiosum* [22]. Members of the genus *Aeromicrobium* are Gram-reaction-positive, non-endospore-forming rods or cocci and are characterized chemotaxonomically by having a tetrahydrogenated menaquinone with nine isoprene units [MK-9(H4)] as the predominant respiratory quinone, and 10-methyl $C_{18:0}$, $C_{16:0}$ and both or either of $C_{18:1}$ $\omega 9c$ and $C_{16:0}$ 2-OH as the major cellar fatty acids [25]. At the time of writing, the genus *Aeromicrobium* comprises 12 recognized species (http://www.bacterio.net). Strains of the genus are commonly isolated from various sources, including soil [4, 10], marine environment [11], Pu'er tea [17], human stools [18], and air [23].

In this study, we describe the taxonomic characterization of new Gsoil 137^T which appeared to be a member of the genus *Aeromicrobium*.

Materials and Methods

Isolation of Bacterial Strain

Strain Gsoil 137^T was isolated from soil of a ginseng field of Pocheon province in South Korea. The soil samples were

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✉ Kang Duk Choi
wandra@hknu.ac.kr

✉ Wan-Taek Im
kdchoi999@gmail.com

[1] Department of Biotechnology, Hankyong National University, 327 Chungang-no, Anseong-si, Kyonggi-do 17579, Republic of Korea

[2] Center for Genetic Information, Graduate School of Bio and Information Technology, Hankyong National University, 327 Chungang-no, Anseong-si, Kyonggi-do 17579, Republic of Korea

collected from different places and thoroughly suspended with 50 mM phosphate buffer (pH 7.0), and the suspensions were spread on R2A agar plates after serial dilution with 50 mM phosphate buffer (pH 7.0). The plates were aerobically incubated at 30 °C for two weeks. Single colony was purified by transferring onto new R2A agar plates and was incubated at 30 °C. Gsoil 137^T was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20%, v/v) at − 80 °C. The strain Gsoil 137^T was deposited to the Korean Collection for Type Cultures (=KCTC 19130^T), German Collections of Microorganisms and Cell Cultures (=DSM 17940^T), and the Culture Collection of the University of Gothenburg (=CCUG 52475^T).

Physiological, Morphological, and Biochemical Characteristics

The Gram reaction was determined using the non-staining method using 3% KOH, as described previously [2]. Cell morphology was examined by a scanning electron microscope (Hitachi SU-3500), using cells grown for 2 days at 30 °C on R2A agar medium. Catalase and oxidase tests were performed as outlined by Cappuccino and Sherman [3]. Biochemical phenotypic tests were carried out using API 20E, API ID 32GN, 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 plates with 1M HCl), casein, and starch were performed and evaluated after 5 days [1]. Growth at different temperatures (4, 10, 18, 30, 37, 42, and 45 °C) and various pH values (pH 3.5–10.0 at intervals of 0.5 pH units) was assessed after 5 days of incubation. The following buffers (final concentration, 20 mM) were used to adjust the pH of R2A broth: acetate buffer was used for pH 3.5–5.5, phosphate buffer was used for pH 6.0–8.0, and Tris buffer was used for pH 8.5–10.0. Salt tolerance was tested on nutrient medium supplemented with 1–10% (w/v at intervals of 1% unit) NaCl after 5 days of incubation. Growth on trypticase soy agar (TSA, BD) and MacConkey agar (BD) was also evaluated at 30 °C.

Phylogenetic Tree Construction and Determination of DNA G+C Content (mol%)

For phylogenetic analysis of strain Gsoil 137^T, DNA was extracted using a genomic DNA extraction kit (Solgent Co. Ltd, Korea). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer set (800R, 1492R, 27F, and 518F) and the purified PCR products were sequenced by Solgent Co. Ltd. (Daejeon, South Korea) as described previously [9]. Almost full length of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank and EzTaxon-e server (http://www.ezbiocloud.net/eztaxon). Multiple sequence alignments were performed by Clustal X program [24]. Gaps were edited in the BioEdit program [7]. Evolutionary distances were calculated using the Kimura two-parameter model [12]. Phylogenic trees were constructed using a neighbor-joining method [19] and maximum-parsimony [6] using the MEGA 6 Program [21] with bootstrap values based on 1000 replications [5].

For the measurement of DNA G+C content, genomic DNA of the novel strain was extracted and purified as described by Moore and Dowhan [16], enzymatically degraded into nucleosides, and determined as described previously [14] using a reverse-phase HPLC.

Chemotaxonomic Analysis

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum conditions, and reextracted in *n*-hexane/water (1:1, v/v). The crude n-hexane–quinone solution was purified using Sep-Pak Vac cartridges silica (Waters) and subsequently analyzed by HPLC as previously described [8]. Cellular fatty acid profiles were determined for strains grown on R2A agar for 48 h. 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 [20]. The presence of diaminopimelic acid (DAP) isomers in the cell wall peptidoglycan was determined using thin-layer chromatography after hydrolysis with 6 N HCl at 100 °C for 18 h as previously described [13].

Nucleotide Sequence Accession Numbers

The 16S rRNA gene sequence of strain Gsoil 137^T determined in this study was deposited in NCBI GenBank/EMBL/DDBJ under the accession number AB245395. The digital protologue database (DPD) number for the strain Gsoil 137^T is TA00193.

Results and Discussion

Morphological and Phenotypic Characteristics

Colonies of strain Gsoil 137^T grown on R2A agar plates for 2 days at 30 °C were convex and creamy colored, which grow well on R2A agar medium, whereas weakly grow on nutrient and Luria–Bertani (BD) agar media, but did not grow on trypticase soy agar (BD), potato-dextrose agar (BD), DNase agar (BD), and MacConkey agar. Strain Gsoil

Table 1 Physiological and biochemical characteristics between strain Gsoil 137^T and closely related species of the genus *Aeromicrobium*

Characteristics	1	2	3	4
Cell morphology	Long rods	Rods[a]	Cocci[b]	Rods[c]
Salinity	0.5–4.5	0–7[a]	0–3[b]	0–0.5[c]
Temperature range (°C)	20–30	10-42[a]	ND	15-30[c]
pH range	5.0–9.0	5.0–10.0[a]	5.0–8.5[b]	5.0–8.5[c]
Oxidase/catalase	−/−	−/+[a]	+/+[b]	−/−[c]
Hydrolysis of				
Gelatin	+	−	+	−
Esculin	−	w	+	−
PNPG	−	−	+	+
Reduction of nitrate	−	−	+	−
Enzymes activity				
Alkaline phosphatase	−	+	+	w
Lipase	−	+	−	−
Cystine arylamidase	w	+	+	w
α-Chymotrypsin	−	w	+	w
α-Glucosidase	−	+	+	−
β-Glucosidase	−	+	w	−
Arginine	+	−	−	−
Lysine decarboxylase	+	−	−	−
H_2S production				
Acetoin	+	+	−	−
Assimilation of				
Citrate	+	w	w	−
D-Glucose	−	−	+	−
Mannitol	+	−	−	−
Sucrose	+	−	−	−
Amygdalin	+	−	−	−
Arabinose	+	−	w	w
L-Rhamnose	+	−	+	−
D-Ribose	+	+	−	−
D-Maltose	+	−	−	+
Suberic acid	−	+	−	+
Sodium malonate	−	+	−	−
Lactic acid	+	+	−	−
L-Alanine	+	+	−	+
L-Serine	+	w	−	−
L-Fucose	+	−	−	−
Propionic acid	w	−	w	−
Valeric acid	w	−	w	−
L-Histidine	−	+	−	+
Potassium 2-ketogluconate	+	+	−	−
L-Proline	+	+	−	−
DNA G+C mol %	70.3	65.9[a]	66.8[b]	65.5[c]
Major menaquinone	MK-8 (H_4)	MK-9 (H_4)	MK-9 (H_4)	MK-9 (H_4)

In the API kit system (ZYM, 20NE, and 32GN), all strains were positive for esterase, esterase lipase, leucine arylamidase, valine arylamidase, trypsin, acid phosphatase inositol, D-saccharose, glycogen, and naphthol-AS-BI-phosphohydrolase and negative for H_2S production, urea hydrolysis, indole production, ornithine decarboxylase, sorbitol, β-galactosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, trisodium citrate, capric acid, salicin, D-melibiose, D -sorbitol, potassium-5-ketogluconate, 3-hydroxybenzoic acid, sodium acetate, itaconic acid, and *N*-acetyl-glucose. +, positive; -, negative; w, weak positive

1 Gsoil 137^T, *2 A. halocynthiae* JCM 15749^T, *3 A. ginsengisoli* KCTC 19207^T, *4 A. panaciterrae* KCTC 19131^T

API kit tests for all strains were conducted under the same conditions used in this study. [a,b,c]data taken from Kim et al. [11], Kim et al. [10], and Cui et al. [4], respectively

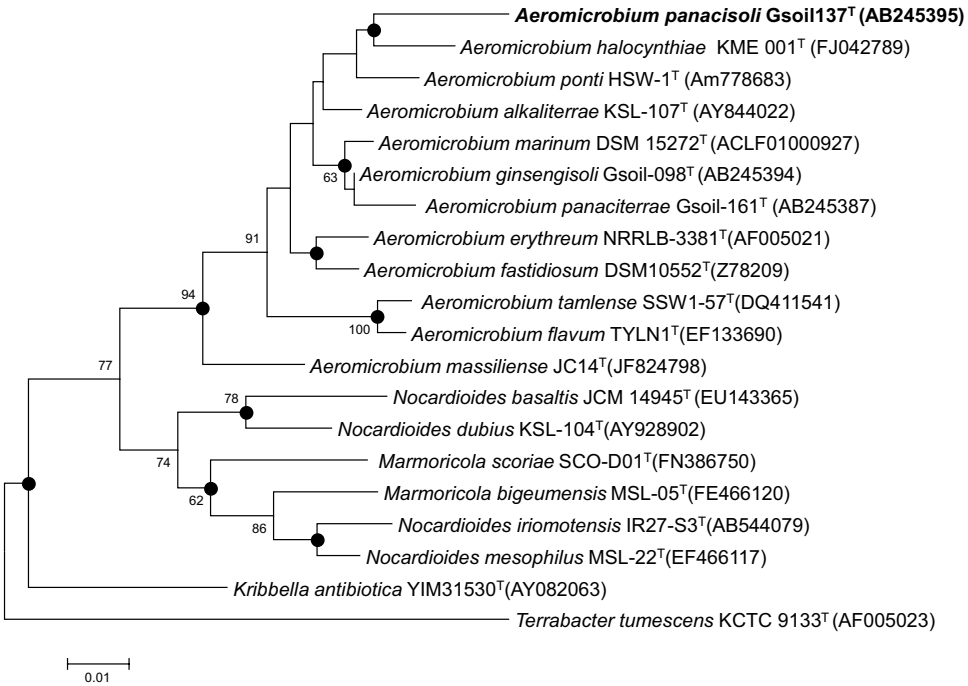

Fig. 1 Phylogenetic tree constructed from a comparative analysis of 16S rRNA gene sequences showing the relationships of strain Gsoil 137^T with other related species. The tree was made using the maximum-likelihood method with two-parameter distance matrix and complete deletion. Dots indicate generic branches that were also recovered using maximum-parsimony and neighbor-joining algorithms. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at the branch points. Bar, 0.01 substitutions per single nucleotide position. *Terrabacter tumescens* KCTC 9133^T (AF005023) was used as an outgroup

137^T was able to grow at 10–30 °C, but not at 4, 35, and 37 °C. Furthermore, the physiological characteristics of strain Gsoil 137^T are summarized in the species description and comparison of selective characteristics with related type strains is shown in Table 1.

Phylogenetic and DNA G+C Content Analysis

The almost complete 16S rRNA gene sequence of strain Gsoil 137^T (1470 nt) was determined and subjected to comparative analysis. Based on the EzTaxon-e analysis, the isolate was assigned to the genus *Aeromicrobium* with the highest sequence similarity to *Aeromicrobium ginsengisoli* KCTC 19207^T (96.7%). The phylogenetic study based on the neighbor-joining, maximum-likelihood, and maximum-parsimony methods confirms that strain Gsoil 137^T clustered within the genus *Aeromicrobium* and form a monophyletic clade with *Aeromicrobium halocynthiae* JCM 15749^T (Fig. 1), well separated from the species of genera *Nocardioides, Marmoricola*, and others.

On the basis of 16S rRNA gene sequence similarity analysis and phylogenetic inference, *Aeromicrobium halocynthiae* JCM 15749^T, *Aeromicrobium ginsengisoli* KCTC 19207^T, and *Aeromicrobium panaciterrae* KCTC 19131^T were selected for comparative study.

The G+C content of genomic DNA of strain Gsoil 137^T was 70.3 mol%.

Chemotaxonomic Characteristics

Many species of the genus *Aeromicrobium* have MK-9(H_4) as the predominant quinone; however, some species of the genus *Aeromicrobium* also possess menaquinone of the MK-7 (H_4) and MK-8 (H_4) types. The menaquinones possessed in strain Gsoil 137^T were MK-8 (H_4) and Mk-7 (H_4) types. The cell wall peptidoglycan of strain Gsoil 137^T contained LL-DAP. The major cellular fatty acids of strain Gsoil 137^T were mainly composed of $C_{14:0}$ (8.5%) $C_{16:0}$ (34.4%), $C_{18:1}$ $\omega 9c$ (21.6%), and $C_{16:1}$ $\omega 7c/C_{15:0}$ [(summed feature 4) 11.3%], which were similar to those of *Aeromicrobium* species (Table 2). The high amount of $C_{14:0}$ (8.5%) and summed feature 4 (11.3%) along with qualitative and quantitative differences in cellular fatty acid analysis distinguishes strain Gsoil 137^T from those of *Aeromicrobium* species (Table 2).

Taxonomic Conclusions

In summary, the characteristics of strain Gsoil 137^T are consistent with descriptions of the genus *Aeromicrobium* with regard to morphological, biochemical, and chemotaxonomic properties. However, on the basis of phylogenetic distance from type strains of *Aeromicrobium* species indicated by 16S rRNA gene sequence similarities and the combination of unique phenotypic characteristics (Table 1), strain Gsoil 137^T represents a novel species, for which the name *Aeromicrobium panacisoli* sp. nov is proposed.

Table 2 Fatty acid profiles of strain Gsoil 137^T and related species of the genus *Aeromicrobium*

Fatty acids	1	2	3	4
Saturated				
$C_{10:0}$	–	–	1.9	–
$C_{14:0}$	**8.5**	**1.3**	–	–
$C_{15:0}$	2.6	1	–	4.8
$C_{16:0}$	**34.4**	**30.7**	**33.5**	**15.1**
$C_{17:0}$	1.8	2.3	2.9	**6.1**
$C_{18:0}$	5.3	6.4	13.3	tr
Unsaturated				
$C_{17:1}$ *ω8c*	4.0	1.8	–	5.1
$C_{18:1}$ *ω9c*	**21.6**	**37.2**	**11.2**	**4.7**
Anteiso-$C_{11:0}$	2.1	–	–	–
Anteiso-$C_{13:0}$	1.0	–	–	–
Hydroxy fatty acids				
$C_{15:0}$ 2-OH	–	tr	–	2.1
$C_{16:0}$ 2-OH	3.0	1.2	5.8	11.3
$C_{17:0}$ 2-OH	–	–	–	3.6
Summed feature[a]				
4. $C_{16:1}$ *ω7c*/$C_{15:0}$ iso 2-OH	**11.3**	**3.2**	**3.7**	**7.4**
Methyl ester				
$C_{16:0}$ 10-methyl	2.8	–	–	12.3
$C_{17:0}$ 10-methyl	tr	–	3.2	10.3
$C_{18:0}$ 10-methyl	1.4	18.7	22.3	16.3

1 Gsoil 137^T, *2 A. halocynthiae* JCM 15749^T, *3 A. ginsengisoli* KCTC 19207^T, *4 A. panaciterrae* KCTC 19131^T

All strains were cultured on R2A agar medium for 48 h at 30 °C. Fatty acids amounting to < 0.5% of the total fatty acids in all strains are not listed. Major fatty acids are shown in bold. tr, trace amounting (tr > 1%); –, not detected

[a]Summed features represent groups of two or three fatty acids that could not be separated by gas chromatography (GLC) with the MIDI system. Summed features consist of 4, $C_{16:1}$ *ω7c*/$C_{15:0}$ iso 2-OH

Description of *Aeromicrobium Panacisoli* sp. Nov

Aeromicrobium 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 type strain).

Cells are Gram positive, strictly aerobic, non-spore-forming, non-motile, and longer rod shaped (1.2–2.0 μm in diameter and 2.0–5 μm in length). Colonies grown on R2A agar are circular, creamy colored, and 0.5–1.5 mm in diameter. Growth occurs at 10–30 °C in the presence of 0.5–4.5% NaCl (w/v) and at pH 6–9. Optimum growth occurs at 30 °C and pH 6.0–7.0 in the absence of NaCl. Catalase and oxidase activities are negative. Gsoil 137^T grow well on R2A agar medium and grow weakly on nutrient agar (NA, BD) and Luria–Bertani agar (LB, BD). The isolate does not grow on potato-dextrose agar (PDA, BD), MacConkey agar (BD), and DNase agar (BD). In the API kit system, the strains were positive for L-rhamnose, *N*-acetyl-glucose, D-ribose, inositol, D-saccharose, D-maltose, lactic acid, L-alanine, glycogen, L-serine, D-mannitol, D-glucose, L-fucose, potassium 2-ketogluconate, esterase, esterase lipase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, arginine dihydrolase, lysine decarboxylase, citrate, acetoin production, gelatin hydrolysis, mannitol, sucrose, amygdalin, and arabinose. List of all negative traits of commercial kits is shown in Table S1. MK-8 (H_4) predominant menaquinone and $C_{14:0}$, $C_{16:0}$, $C_{18:1}$ *ω9c* and summed feature 4 (comprising $C_{16:1}$ *ω7c*/$C_{15:0}$ iso 2-OH) are the major components of cellular fatty acids. The DNA G+C content of genomic DNA is 70.3 mol%. The cell wall peptidoglycan of strains Gsoil 137^T contains LL-DAP.

The type strain Gsoil 137^T (= KCTC 19130^T = DSM 17940^T = CCUG 52475^T) was isolated from soil of a ginseng field of Pocheon province, South Korea.

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