Arachidicoccus ginsenosidivorans sp. nov., with ginsenoside-converting activity isolated from ginseng cultivating soil

Muhammad Zubair Siddiqi, 1, 2 Zubair Aslam 3 and Wan-Taek Im 1, 2, *

Abstract
A Gram-reaction-negative, catalase- and oxidase-positive, aerobic, non-motile, light yellow and rod-shaped bacterium (designated Gsoil 809T) isolated from soil of ginseng field, was characterized by a polyphasic approach to clarify its taxonomic position. Strain Gsoil 809T was observed to grow optimally at 30°C and at pH 7.0 on nutrient agar medium. Strain Gsoil 809T possessed β-glucosidase activity, which was responsible for its ability to transform protopanaxatriol-type ginsenoside Rg1 to ginsenoside Rh1. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain Gsoil 809T belongs to the genus Arachidicoccus of the family Chitinophagaceae and was most closely related to Arachidicoccus rhizosphaerae Vu-144T (98.1 % 16S rRNA gene sequence similarity). The DNA G+C content was 39.4 mol%. The DNA–DNA hybridization value between strain Gsoil 809T and A. rhizosphaerae Vu-144T was 41.27±1.03 %. The major polar lipids were phosphatidylethanolamine and an unknown polar lipid. The predominant quinone was MK-7. The major fatty acids were iso-C15:0, iso-C15:1 G, iso-C17:0 3-OH and summed feature 3, which supported the affiliation of Gsoil 809T to the genus Arachidicoccus. Strain Gsoil 809T contained homospermidine as the major polyamine. Moreover, the physiological and biochemical test results and low DNA–DNA relatedness value allowed the phenotypic and genotypic differentiation of strain Gsoil 809T from recognized species of the genus Arachidicoccus. Therefore, strain Gsoil 809T represents a novel species of the genus Arachidicoccus, for which the name Arachidicoccus ginsenosidivorans sp. nov. is proposed. The type strain is Gsoil 809T (=KCTC 22820T=JCM 30984T).

The genus Arachidicoccus was recently described by Madhaiyan et al. [1] based on a single species description, Arachidicoccus rhizosphaerae. Phylogenetically, the genus is a member of the phylum Bacteroidetes and forms an evolutionary lineage within the family Chitinophagaceae. At the time of writing, the genus Arachidicoccus comprises only one recognized species, A. rhizosphaerae (www.bacterio.net). Cells of the member of the genus are Gram-stain-negative, non-motile, non-flagellated and non-spore-forming [1]. The affiliate of the genus is negative for Flexirubin-type reactions. The predominant quinone was menaquinone MK-7, the major polar lipid was phosphatidylethanolamine (PE), and iso-C15:0, iso-C15:1 G, iso-C17:0 3-OH and summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c) were the main cellular fatty acids. Homospermidine is the major polyamine of the representative of the genus Arachidicoccus of the family Chitinophagaceae.

In this study, we describe a novel bacterial strain, designated as Gsoil 809T, which was isolated from soil of a ginseng field in Pocheon province, Republic of Korea. The soil sample was thoroughly suspended with sterilized water, following serial dilution, and was then spread onto R2A agar medium (Difco) and incubated at 30°C for 2 weeks. A single colony was purified by subculture. Strain Gsoil 809T was routinely cultured on R2A agar at 30°C and maintained as a glycerol suspension (25 %, v/v) at −80°C.

Genomic DNA of strain Gsoil 809T was extracted with a commercial genomic DNA extraction kit (Solgent). The bacterial universal primer sets 800R, 1492R, 27F and 518F were used to amplify the 16S rRNA gene sequence [2]. The purified PCR product was sequenced by Genotech according to Im et al. [3], and the nearly full-length sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database (www.ncbi.nlm.nih.gov).

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**Keywords:** Arachidicoccus ginsenosidivorans; 16S rRNA gene sequence; polyphasic taxomony; biotranformation.

**Abbreviation:** PE, phosphatidylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 809T is EU370955.

Five supplementary figures are available with the online Supplementary Material.
ally performed according to the manufacturer.

Gsoil 809

ZYM, API 20NE and API 32GN (bioM

com-

simony tree was made with Subtree-Pruning-Regrafting (SPR) heuristic method and the gaps were edited with com-

tiate soy agar (TSA), lysogeny broth (LB) agar and MacConkey agar (all from Difco) at 30 °C for 1 week. Production of flexi-

simony tree was made with Subtree-Pruning-Regrafting (SPR) heuristic method and the gaps were edited with com-

complete deletion [9] using the

The almost-complete 16S rRNA gene sequence of strain Gsoil 809

The isolate did not grow on TSA, LB or MacConkey agar,

The Gram reaction was determined using the non-staining method, as described by Buck [12]. Cell motility was deter-

Based on 16S rRNA gene sequence and phylogenetic analy-

sis, A. rhizosphaerae KCTC 22378

Based on 16S rRNA gene sequence and phylogenetic analy-

sis, the genus Arachidicoccus (Fig. 1). Moreover, this relation-

ship was also evident in phylogenetic trees based on the

neighbour-joining and maximum-parsimony methods (Figs

S1 and S2, available in the online Supplementary Material).

The highest degree of 16S rRNA gene sequence similarity determined was to A. rhizosphaerae KCTC 22378

(98.1 %).

The Gram reaction was determined using the non-staining

method, as described by Buck [12]. Cell motility was deter-

mined using hanging-drop method, while cell morphology

was examined with the transmission electron microscope

(SU-3500, Hitachi), using cells grown for 2 days at 30 °C on

NA medium. Oxidase activity was determined using 1 %

(w/v) N,N,N,N-tetramethyl-1,4-phenylenediamine reagent

(bioMérieux). Catalase activity was determined by the pro-

duction of bubbles from 3 % (v/v) H2O2 solution. Hydroly-

sis activity was tested using the following substrates: starch

[NA containing 1 % starch (Difco)], casein [NA containing

2 % skimmed milk (Difco)] [13], aesculin [NA containing

aesculin, 0.3 %, (Sigma), and ferric citrate, 0.2 % (Fluka)],

DNase [DNase agar medium (Sharlau)], Tween 80 (NA

containing 1 % Tween 80 and 0.02 % CaCl2) and CM-cellu-

lose (CM-cellulose [14]), and Voges–Proskauer reactions

were examined as described by Lányi [15]. All tests were

performed and evaluated after incubation for 2 days at

30 °C. Indole production was tested using 1 % tryptone

broth. Biochemical tests in the commercial systems API

ZYM, API 20NE and API 32GN (bioMérieux) were gen-

erally performed according to the manufacturer's instruc-

tions. The API ZYM test strips were read after 4 h of incubation at

37 °C, and the other API strips were examined after 2 days

at 30 °C. Growth at different temperatures (4, 10, 15, 25, 30,

37 and 40 °C) and pH (pH 4–10 at intervals of 0.5 and 1 pH

units) was assessed after incubation for 5 days at 30 °C. The

following buffers (final concentration, 50 mM) were used to

adjust the pH of nutrient broth: acetate buffer for pH 4.0–

5.5, phosphate buffer for pH 6.0–8.0, and Tris buffer for pH

8.5–10.0. Salt tolerance was evaluated on nutrient agar

medium supplemented with 0–8 % (w/v, at intervals of 0.5

and 1 %) NaCl and growth was assessed after incubation

for 7 days at 30 °C. Growth on different media was also tested by using nutrient agar (NA), trypticase soy

agar (TSA), lysogeny broth (LB) agar and MacConkey agar

(all from Difco) at 30 °C for 1 week. Production of flexi-

rubin-type pigments was determined by the reversible colour

shift to red, purple or brown when yellow or orange colonies

are covered with an aqueous solution of 20 % KOH [16].

Ginsenosides Rb1, Rc, Re, Rg1, F2, C-Mc, Rh1 and com-

pound K were purchased from Nanjing Zelang Medical

Technology (Nanjing, China). The reaction mixture (each

ginsenoside in separate endoperox tube), containing 200 µl

of 2 mg ml
to ginsenosides Re and Rg1 and 200 µl of a bacterial

suspension inoculated in nutrient broth, was incubated for

1 day at 150 r.p.m. and 30 °C. After 24 h, a 50 µl aliquot

was taken and extracted with an equal volume of 80 %

water-saturated n-butanol, and subjected to TLC analysis.

TLC was performed using 60F254 silica gel plates (Merck)

with CHCl3/CH3OH/H2O (65:35:10, by vol., lower phase)

as the solvent. The spots on the TLC plates were detected by

spraying with 10 % (v/v) H2SO4 followed by heating at

110 °C for 5 min.

Cells of strain Gsoil 809

were Gram-reaction-negative, aer-

obic, non-motile, non-spore-forming, and rod-shaped as

shown in Fig. S3. Colonies of strain Gsoil 809

grown on nutrient agar medium were circular, convex, opaque and

light yellow after incubation for 48 h at 30 °C. Cells were

negative for the production of flexirubin-type pigments.

The isolate did not grow on TSA, LB or MacConkey agar,

but weakly grew on R2A and DNase agar at 30 °C. Strain

Gsoil 809

was negative for the Voges-Proskauer reaction and

hydrolysis of starch, casein, Tween 80, cellulose and

DNase, but positive for the hydrolysis of aesculin. Physio-

logical characteristics of strain Gsoil 809

are summarized in the species description, and a comparison of selective

characteristics of the isolated strain and related type strain

is given in Table 1. After incubation for 1 day at 30 °C, the biotransformation of the ginsenosides by strain Gsoil 809

showed that ginsenoside Re was not transformed and gins-

enoside Rg1 was completely bioconverted into ginsenoside

Rh1).

To analyse the DNA G+C content of strain Gsoil 809

the genomic DNA was extracted and purified as described by

Moore and Dowhan [17], and was degraded enzymatically

into nucleosides, then the DNA G+C content was deter-

mined as described by Mesbah et al. [18] using a reverse-

phase HPLC. Strain Gsoil 809

was examined for polar lipid contents as described by

Minnikin et al. [19].
One hundred milligrams of freeze-dried cells were dissolved in methanol/water (10:1, v/v) and stirred well. Then, 2 ml petroleum ether was added. After centrifugation at 2000 r.p.m. for 5 min, the upper layer was discarded, and the lipid residues were taken twice with the first extraction as chloroform/methanol/water (90:100:30, by vol.) and the second extraction as (50:100:40, by vol.). Chloroform and 0.3 % NaCl (1.3 ml each) solution were added to the combined supernatant, mixed thoroughly, and centrifuged. The upper layer was discarded with a pasteur pipette and only the bottom layer was used. Polar lipids were dissolved in chloroform/methanol (2:1, v/v), and the samples were spotted on the corner of TLC Kiesel gel 60F254 (Merck) plates (10 × 10 cm) for two-dimensional TLC. The polar lipids were developed in the first direction by using chloroform/methanol/water (65:25:4, by vol.); while the second

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**Fig. 1.** Phylogenetic relationship between strain Gsoil 809T and other related species of the family Chitinophagaceae. The tree was reconstructed using the maximum-likelihood method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) >60 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with maximum-parsimony and neighbour-joining algorithms. Thermoflavifilum aggregans P373T (GenBank accession number AM749771) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.
**Table 1.** Differential characteristics between strain Gsoil 809\(^T\) and the type strain of the related species of the genus *Arachidicoccus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1 Isolation source</th>
<th>2 Cell morphology</th>
<th>3 Temperature range (°C)</th>
<th>4 pH range</th>
<th>5 Salinity range for growth (% NaCl, w/v)</th>
<th>6 Enzyme activity</th>
<th>7 Carbon utilization of</th>
<th>8 DNA G+C content (mol%)</th>
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</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Ginseng field</td>
<td>Rhizosphere</td>
<td>10–37</td>
<td>5–7</td>
<td>0–4</td>
<td>Arginine dihydroase</td>
<td>D-Glucose</td>
<td>39.4</td>
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<td></td>
<td>α-Chymotrypsin</td>
<td>D-Mannose</td>
<td>43.1*</td>
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<td></td>
<td>Esterase lipase</td>
<td>Maltose</td>
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<td>Propionic acid</td>
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<td>Glycogen</td>
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<td></td>
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<td>Sarcin</td>
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<td></td>
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<td>L-Fucose</td>
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<td>D-Sorbitol</td>
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<td></td>
<td>Histidine</td>
<td>1–Proline</td>
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<td>DNA G+C content (mol%)</td>
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</table>

*Data for *A. rhizosphaerae* KCTC 22378\(^T\) from Madhaiyan et al. [1].

The DNA G+C content of strain Gsoil 809\(^T\) was 39.4 mol%, which was similar to that of *A. rhizosphaerae* KCTC 22378\(^T\) (Table 1). The main detected polar lipids of strain Gsoil 809\(^T\) were phosphatidylethanolamine (PE) and one unknown polar lipid (L1); the minor polar lipids were five unknown polar lipids (L2–L6) and one unknown amino lipid (AL) (Fig. S4). Based on the polar lipid analysis, strain Gsoil 809\(^T\) shares major polar lipid PE with the recently described species of the family *Chitinophagaceae* [1, 23–27]. Comparative analysis of strain Gsoil 809\(^T\) with *A. rhizosphaerae* KCTC 22378\(^T\) indicates that both strains share PE and L1 as major polar lipids. Correspondingly, the differences of polar lipids between strain Gsoil 809\(^T\) and *A. rhizosphaerae* KCTC 22378\(^T\) were specified by marking the spots in Fig. S4. The major respiratory quinone was MK-7. The fatty acid profile of strain Gsoil 809\(^T\) was compared with the type species of the genus *Arachidicoccus*. The major fatty acids of strain Gsoil 809\(^T\) were iso-C\(15:0\) (42.7 %), iso-C\(15:1\) G (14.9 %), iso-C\(17:0\) 3-OH (20.7 %) and summed feature 3 (comprising C\(16:1\)ω7t and/or C\(16:1\)ω6c; 9.3 %), which is a typical profile of members of the genus *Arachidicoccus* [1]. However, some qualitative and quantitative differences in the fatty acids distinguished strain Gsoil 809\(^T\) from *A. rhizosphaerae* KCTC 22378\(^T\) (Table 2). The polyamines of strain Gsoil 809\(^T\) were homospermidine (major) and methylbenzoate (minor). The DNA–DNA relatedness value between strain Gsoil 809\(^T\) and *A. rhizosphaerae* KCTC 22378\(^T\) was 41.27±1.03 %; this hybridization value is below the 70 % threshold proposed for species delineation [28], and shows that strain Gsoil 809\(^T\) represents a distinct genomic species of the genus *Arachidicoccus*.

On the basis of the phylogenetic, genotypic, chemotaxonomic and physiological and biochemical data, it is appropriate to conclude that strain Gsoil 809\(^T\) should be assigned to the genus *Arachidicoccus* as the type strain of a novel species, for which the name *Arachidicoccus ginsenosidivorans* sp. nov. is proposed.

**DESCRIPTION OF ARACHIDICOCUS GINSENOsidIVORANS SP. NOV.**


Cells are Gram-reaction-negative, aerobic, non-motile and rod shaped (width, 0.4–1 μm; length, 1.5–3 μm). Colonies grown at 30 °C on nutrient agar for 2 days are light yellow, smooth, convex, opaque, circular with regular margins, and methylated and extracted according to the described method of the Sherlock Microbial Identification System (MIDI). The fatty acids analysed by a gas chromatograph (Hewlett Packard 6890) were identified by the Microbial Identification software package based on Sherlock Aerobic Bacterial Database (TSBA60) [21]. Polyamines were extracted and analysed according to Schenkel et al. [22].
Table 2. Cellular fatty acid contents (%) of strain Gsoil 809T and the type strain of the phylogenetically related species of the genus Arachidicoccus

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Saturated</td>
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<tr>
<td>C14:0</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>C16:0</td>
<td>3.8</td>
<td>3.7</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Branched</td>
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<tr>
<td>iso-C15:0</td>
<td>0.1</td>
<td>–</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td>–</td>
<td>0.1</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>42.7</td>
<td>39.5</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>2.7</td>
<td>2.8</td>
</tr>
<tr>
<td>iso-C15:1</td>
<td>14.9</td>
<td>17.1</td>
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<tr>
<td>anteiso-C15:0</td>
<td>0.9</td>
<td>0.5</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>–</td>
<td>0.5</td>
</tr>
<tr>
<td>iso-C17:0</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>iso-C17:1</td>
<td>20.7</td>
<td>22.8</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
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<tr>
<td>C16:0 2-OH</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>C16:0 3-OH</td>
<td>3.8</td>
<td>4.4</td>
</tr>
<tr>
<td>C17:0 2-OH</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>Summed feature*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>9.3</td>
<td>7.5</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two or three fatty acids that could not be separated by gas chromatography (GLC) with the MIDI system. Summed feature 3 consists of C16:0 1ω7c and/or C16:1ω6c.

2–3.5 mm in diameter. Growth occurs at 10–37 °C and pH 5–7 with 0–4 % NaCl (w/v). Optimum growth occurs at 30 °C and pH 7.0 with 0–1 % NaCl (w/v). Indole production is negative. In the API kits (API 20 NE, 32 GN and API ZYM), positive for the reduction of nitrate, and alkaline phosphatase, esterase, leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, napthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase activities. L-Rhamnose, sucrose, L-arabinose and melibiose are utilized. Negative for lipase, L-arabinose, α-mannosidase, L-tryptophan, urease and gelatin hydrolysis. Does not utilize maltose, D-ribose, inositol, itaconic acid, suiceric acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, l-serine, 6-mannitol, capric acid, valeric acid, trisodium citrate, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid, potassium glutonate, adipic acid, malic acid and phenylacetic acid. MK-7 is the predominant respiratory quinone and iso-C15:0, iso-C15:1 G, iso-C17:0 3-OH and summed feature 3 (comprising iso-C16:1ω7c and/or C16:1ω6c) are the major cellular fatty acids. The polar lipid profile is composed of PE, six unknown polar lipids (L1–L6) and an unknown aminolipid. TLC analysis shows that the type strain Gsoil 809T converts major ginsenoside Rg1 (PPT-type) to minor ginsenoside Rh1, while the ginsenoside Re is not changed (Fig. S5).

The type strain, isolated from ginseng cultivating soil, Republic of Korea, is Gsoil 809T (=KCTC 22820T=JCM 30984T). The G+C content of the genomic DNA of the type strain is 39.4 mol%. The polyamines are homospermidine and methylbenzoate.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References


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