Anseongella ginsenosidimutans gen. nov., sp. nov., isolated from soil cultivating ginseng

Muhammad Zubair Siddiqi,1,2 Qingmei Liu,1,2 Myung-Suk Kang,3 Minseok S. Kim4 and Wan-Taek Im1,2

Correspondence Wan-Taek Im wandra@hknu.ac.kr

1Department of Biotechnology, Hankyoung National University, 327 Chungang-no Anseong-si, Kyonggi-do 456-749, Republic of Korea
2Center for Genetic Information, Graduate School of Bio and Information Technology, Hankyoung National University, 327 Chungang-no Anseong-si, Kyonggi-do 456-749, Republic of Korea
3Microorganism Resources Division, National Institute of Biological Resources, Incheon 404-708, Republic of Korea
4Department of Biomedical Engineering, Konyang University, 121 Daehak-ro, Nonsan, Chungnam, Republic of Korea

A Gram-stain-negative, rod-shaped, non-spore-forming, oxidase and catalase-positive, strictly aerobic bacterium, designated strain Gsoil 524T, was isolated from the soil of a ginseng field and subjected to polyphasic taxonomic analysis. Phylogenetic analysis, based on the 16S rRNA gene sequence, placed Gsoil 524T in a distinct lineage in the family Sphingobacteriaceae, sharing 87.2–88.0 % sequence similarity with members of the closely related genera Pedobacter, Mucilaginibacter and Solitalea. Strain Gsoil 524T contained MK-7 as the predominant quinone, and iso-C15 : 0, iso-C17 : 0 3-OH, summed feature 3 (C16 : 1 ω7c and/or C16 : 1 ω6c) and C16 : 1 ω5c as the major fatty acids. Strain Gsoil 524T could be distinguished from the other members of the family Sphingobacteriaceae by a number of chemotaxonomic and phenotypic characteristics. The major polar lipids in strain Gsoil 524T were phosphatidylethanolamine and two unidentified polar lipids. Compared with the standard and reference strains unidentified sphingolipid was also found. Based on this polyphasic taxonomic analysis, strain Gsoil 524T represents a novel species within a novel genus, for which the name Anseongella ginsenosidimutans gen. nov., sp. nov. is proposed. The type strain of Anseongella ginsenosidimutans is Gsoil 524T (=KACC 14636T=KCTC 22261T=LMG 24494T).

The family Sphingobacteriaceae, which belongs to the phylum Bacteroidetes, class Sphingobacteria and order Sphingobacteriales was first proposed by Steyn et al. (1998). The members of Sphingobacteriaceae have been mostly isolated from soil and water. At the time of writing the family Sphingobacteriaceae contains twelve genera, but only ten genera with validly published names: Mucilaginibacter (Pankratov et al., 2007), Nubsella (Asker et al., 2008), Olivibacter (Ntougias et al., 2007), Parapedobacter (Kim et al., 2007), Pedobacter (Steyn et al., 1998), Pseudosphingobacterium (Vaz-Moreira et al., 2007), Solitalea (Weon et al., 2009), Sphingobacterium (Yabuuchi et al., 1983) and the genera Arcticibacter (Prasad et al., 2013) and Pseudopedobacter (Cao et al., 2014) with recently published names (http://www.ncbi.nlm.nih.gov/Taxonomy). Sphingobacteriaceae differs from the family Flavobacteriaceae due to the presence of sphingolipids, MK-7 being the major quinone, the relatively high G+C contents and the different fatty acids contents.

The soil of a ginseng field of pH 5.6 was collected from Pocheon province, Republic of Korea. The soil was thoroughly suspended with sterilized water, following serial dilution it was then spread onto R2A agar medium (BD). The plates were incubated at 30 °C for 14 days. Single colonies were purified by subculture. Strain Gsoil 524T was found and then it was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (25 %, v/v) at −70 °C.

Genomic DNA was extracted with a commercial genomic DNA extraction kit (Solgent) and PCR-mediated amplification of the 16S rRNA gene and sequencing of the purified
PCR product were carried out according to Im et al. (2010). Full sequences of the 16S rRNA gene were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database or http://www.ezbiocloud.net/eztaxon (Kim et al. 2012). Multiple alignments were performed using the CLUSTAL X program (Thompson et al., 1997). Gaps were edited in the BioEdit program (Hall, 1999). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed by using the neighbour-joining, maximum-likelihood and maximum-parsimony methods (Fitch, 1971; Saitou & Nei, 1987) using the MEGA 6 program (Tamura et al., 2012). Multiple alignments were performed using the MEVGA6 program (Tamura et al., 2013) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Nearly complete 16S rRNA gene sequences of strain Gsoil 524T (1457 nt) were determined and subjected to comparative analysis. Sequence similarities were determined using the EzTaxon-e server (Kim et al., 2012). Phylogenetic analysis of strain Gsoil 524T indicated that it represented a member of the family Sphingobacteriaceae, forming a separate clade within the family Sphingobacteriaceae (Fig. 1). Representative species from all ten genera of the family Sphingobacteriaceae were included in the phylogenetic tree. Strain Gsoil 524T showed 16S rRNA gene similarities of less than 88.0 % to those of type species of the genera Pedobacter, Solitalea, Mucilaginibacter and others.

For phenotypic and biochemical tests, strain Gsoil 524T was routinely cultivated in R2A medium. Gram-staining, oxidase and catalase activities, hydrolysis of casein, CM-cellulose, DNA, starch, Tween 80 and xylan were determined according to the methods of Smibert & Krieg (1994). Cell morphology was examined by using a scanning electron microscope (Hitachi; SU-3500) with cells grown for 2 days. Cell motility was determined using the hanging drop method. Growth at different temperatures (4, 10, 15, 25, 30, 37 and 42 °C) and various pH values (pH 4–10.0 at intervals of 1.0 pH unit) was evaluated after 5 days incubation at 30 °C. Three different buffers (final concentration, 50 mM) were used to adjust the pH of R2A broth. Acetate buffer was used for pH 4.0–5.5, 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 R2A medium supplemented with 1–10 % NaCl (w/v, at intervals of 1 %) and growth was assessed after 7 days of incubation. Growth on nutrient agar (NA, BD), tryptase soy agar (TSA, BD), LB agar (BD) and MacConkey agar (BD) was also evaluated at 30 °C. Tests in the API ZYM, API 20NE, and API ID 32 GN (bioMérieux) commercial systems were generally performed according to the manufacturer’s instructions. The API ZYM test strip was read after 4 h incubation at 37 °C and the other API strips were examined after 2 days at 30 °C. Ginsenoside biotransformation was carried out as described by Kim et al. (2015).

![Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain Gsoil 524T. Numbers at nodes indicate percentages based on 1000 bootstrap resamplings. Filled circles indicate that the corresponding branches were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.](http://www.microbiologyresearch.org)
Cells were Gram-stain-negative, strictly aerobic, non-motile, non-spore-forming and rod-shaped (Fig. S1, available in the online Supplementary Material). Colonies of strain Gsoil 524 grown on R2A agar were circular, convex, translucent and milky-white after 48 h incubation at 30 °C. Strain Gsoil 524 grew well on R2A medium and weakly on nutrient agar (Difco), but did not grow on LB agar (BD), trypticase soy agar (BD) or MacConkey agar (Difco). Strain Gsoil 524 possessed α-glucosidase activity, which was responsible for its ability to transform ginsenoside Rb1 (one of the dominant active components of ginseng) to ginsenoside Rd.

The genera Pedobacter and Sphingobacterium are members of the family Sphingobacteriaceae (Euzeby, 1997). Production of catalase and oxidase, hydrolysis of aesculin, presence of β-galactosidase, utilization of a large number of carbohydrates and of a few organic acids and amino acids are the common phenotypic characteristics of members of the genera Pedobacter and Sphingobacterium (Steyn et al., 1998; Kim et al., 2006). Chemotaxonomic markers of these genera are the presence of MK-7 and sphingolipids, a genomic DNA G+C content of around 40 mol% and a characteristic fatty acid methyl ester pattern (Steyn et al., 1998; Kim et al., 2006). They also have iso-C₁₅ : 0, iso-C₁₇ : 0 2-OH, iso-C₁₅ : 0 3-OH, C₁₆ : 0, C₁₆ : 1ω7c, C₁₆ : 0 3-OH and ISO-C₁₇ : 0 3-OH as the predominant fatty acids. All these properties were observed in strain Gsoil 524, thus confirming the affiliation of this isolate to the family Sphingobacteriaceae (Steyn et al., 1998). Similar to the physiological characteristics of strain Gsoil 524, they are summarized in the species description and Table 1, which shows a comparison between strain Gsoil 524 and species of the related genera.

Genomic DNA of the novel strain and the two reference strains was extracted and purified, as described by Moore & Dowhan (1995) and enzymically degraded into nucleosides. The G+C content was then determined as described by Mesbah et al. (1989), using a reverse-phase HPLC system (Youngin). Cellular fatty acid profiles were determined using cells grown on R2A agar for 2 days at 30 °C. The cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock

<table>
<thead>
<tr>
<th>Table 1. Differential phenotypic characteristics of Anseongella gen. nov. and related genera of the family Sphingobacteriaceae</th>
</tr>
</thead>
</table>

**Taxa:** 1, *Anseongella 2, Solitalea* (2 species) (data from Weon et al., 2009); 3, *Mucilaginosbacter* (2 species) (data from Pankratov et al., 2007); 4, *Nubella* (Asker et al., 2008); 5, *Olivibacter* (4 species) (Ntougias et al., 2007; Wang et al., 2008; Chen et al., 2013); 6, *Parapedobacter* (2 species) (Kim et al., 2007, 2008); 7, *Pedobacter* (20 species) (Steyn et al., 1998; Shivaji et al., 2005; Vanparys et al., 2005; Gallego et al., 2006; Hwang et al., 2006; Ten et al., 2006b; Baik et al., 2007; Yoon et al., 2006, 2007a, 2007b; Kwon et al., 2007; Muurholm et al., 2007; Oh et al., 2013; Derichs et al., 2014); 8, *Sphingobacterium* (7 species) (Takeuchi & Yokota, 1992; Steyn et al., 1998; Ten et al., 2006a; Mehnaz et al., 2007; Yoo et al., 2007; Liu et al., 2008); 9, *Pseudosphingobacterium* (1 species) (Vaz-Moreira et al., 2007). +, Positive; −, negative; v, variable; ND, no data available; FA, facultatively aerobic; SA, strictly aerobic.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relation to oxygen</td>
<td>SA</td>
<td>V</td>
<td>FA</td>
<td>SA</td>
<td>SA</td>
<td>SA</td>
<td>SA</td>
<td>FA</td>
<td>SA</td>
</tr>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 °C</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>ND</td>
</tr>
<tr>
<td>40 °C</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>&gt;1% NaCl (w/v)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>V</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glucose fermentation</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>V</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>−</td>
<td>+</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>V</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>V</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Malate</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>51.9</td>
<td>37–38.1</td>
<td>42–46</td>
<td>38.6</td>
<td>39.2–45.6</td>
<td>45.6–50.1</td>
<td>36.9–44.2</td>
<td>37.7–44.2</td>
<td>42</td>
</tr>
</tbody>
</table>

*No data available for Pedobacter caeni, Pedobacter africanus or Pedobacter saltans.
†Data only for Parapedobacter koreensis.
‡Data only for three species: Olivibacter soli, Olivibacter ginsengisoli and Olivibacter terrae.
§Data only for Olivibacter sitiensis.
Microbial Identification System (MIDI). They were then identified by capillary GLC, using version 6.0 of the Microbial Identification software package (MIDI) the TSBA60 database (Sasser, 1990) and a 6890 chromatograph (Hewlett Packard). Cell biomass of strain Gsoil 524 for Microbial Identification software package (MIDI) was identified by capillary GLC, using version 6.0 of the Microbial Identification System (MIDI). They were then lyophilized for 24 h and were extracted with chloroform/methanol (2:1, v/v), evaporated under vacuum, and re-extracted in n-hexane/water (1:1, v/v). The crude n-hexane-quinoine solution was purified using a Sep-Pak Vac silica cartridge (Waters) and subsequently analysed by HPLC, as previously described (Hiraishi et al., 1996). The polar lipid contents were extracted from 100 mg of freeze-dried cells, examined by two-dimensional TLC and identified as described by Minnikin et al. (1984). For the analysis of sphingolipids, cell mass of strain Gsoil 524T, Sphingobacterium multi-vorum ATCC 33613T Sphingobacterium canadense LMG 23727T and Sphingobacterium cladoniae KCTC 22613T were harvested from R2A broth after incubation for 2 days at 30 °C. The sphingolipids were extracted and analysed by TLC using the method of Dees et al. (1985).

The G+C content of the novel isolate was 51.9 mol%, which is the highest value in the family Sphingobacteriaceae. The cellular fatty acid profile of strain Gsoil 524T is shown in Table S1. The predominant fatty acids of strain Gsoil 524 were iso-C15:0 (32.1 %), iso-C17:0 3-OH (20.5 %), summed feature 3 [(C16:1ω7c and/or C16:1ω6c) 11.7 %] and C16:0 (9.4 %), iso-C15:0 (6.8 %), which accounted for 80.5 % of the total fatty acids. A distinguishing feature was that strain Gsoil 524T contained considerable amounts of C16:0ω5c (9.4 %), of which there has been found to be less than 5.8 % in the types species of the other genera. The polar lipid profile of strain Gsoil 524T comprised phosphatidylethanolamine, seven unidentified polar lipids, one unidentified aminophospholipid, one unidentified phospholipid and one unidentified aminolipid (Fig. S2). Using D-sphingosine as standard, sphingolipids were detected in strain Gsoil 524T and three reference strains (Sphingobacterium multi-vorum ATCC 33613T Sphingobacterium canadense LMG 23727T and Sphingobacterium cladoniae KCTC 22613T), which is a distinct feature of members of the family Sphingobacteriaceae (Fig. S3). Strain Gsoil 524T contained MK-7 (80 %) as the predominant menaquinone; it also contained MK-6 (15 %) and MK-8 (5 %) as minor menaquinones. On the basis of phylogenetic positioning and phenotypic characteristics, strain Gsoil 524T represents a novel species of a novel genus in the family Sphingobacteriaceae, for which the name Anseongella ginsenosidimutans gen. nov., sp. nov. is proposed.

**Description of Anseongella ginsenosidimutans gen. nov., sp. nov.**

Anseongella (an.seong.el’la. N.L. fem. adj. Anseongella refers to Anseong city in the Republic of Korea, where taxonomic studies of this taxon were performed). Cells are Gram-stain-negative, strictly aerobic rods or elongated rods. The major respiratory quinone is MK-7. The major fatty acids are iso-C15:0, iso-C17:0 3-OH, summed feature 3 (C16:1ω7c and/or C16:1ω6c) and C16:1ω5c. The G+C content of the genome DNA are 51.9 mol%. Phylogenetically, the genus is affiliated to the family Sphingobacteriaceae. The type species is Anseongella ginsenosidimutans sp. nov.

**Acknowledgements**

This work was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment.
Sphingobacteriaceae gen. nov., sp. nov., a zeaxanthin-producing bacterium of the family


