Marmoricola ginsengisoli sp. nov. and Marmoricola pocheonensis sp. nov. isolated from a ginseng-cultivating field

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Two novel actinobacteria, designated strains Gsoil 097T and Gsoil 818T, isolated from soil of a ginseng field, South Korea, were characterized by a polyphasic approach to clarify their taxonomic positions. They were Gram-reaction-positive, aerobic, non-spore-forming and rod-shaped. Phylogenetic analysis based on 16S rRNA gene sequences indicated that both isolates belong to the genus Marmoricola and were related most closely to Marmicola solisilvae KIS18-7T (99.1 and 98.3 % similarity, respectively), Marmicola terrae JOS5-1T (97.9 and 97.9 %), Marmicola scoriae Sco-D01T (97.8 and 97.1 %) and Marmicola aequoreus SST-45T (97.5 and 97.0 %). The G+C content of the genomic DNA was 68.8 and 70.0 mol%, respectively. Both strains were characterized chemotaxonomically as having LL-2,6-diaminopimelic acid in the cell-wall peptidoglycan, MK-8(H4) as the predominant menaquinone and C17:1v6c, C18:1v9c, C18:010-methyl and iso-C16:0 as major fatty acids. These chemotaxonomic data supported the affiliation of both strains to the genus Marmoricola. However, levels of DNA–DNA relatedness between the two strains and closely related type strains of Marmoricola species were less than 30 %. Moreover, the results of physiological and biochemical tests allowed the phenotypic differentiation of strains Gsoil 097T and Gsoil 818T from other Marmoricola species with validly published names. Therefore, the two isolates represent two novel species, for which the names Marmoricola ginsengisoli sp. nov. (type strain Gsoil 097T = KACC 14267T = DSM 22772T) and Marmoricola pocheonensis sp. nov. (type strain Gsoil 818T = KACC 14275T = DSM 22773T) are proposed.

The genus Marmoricola was first described by Urzi et al. (2000) and the description has been emended by Dastager et al. (2008), Lee & Lee (2010) and Kim et al. (2015b). Members of the genus Marmoricola in the family Nocardioidaceae are characterized as Gram-reaction-positive, aerobic and morphologically variable. They contain menaquinone MK-8(H4) as the predominant respiratory quinone, LL-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan and have a high DNA G+C content of 62.9–73.0 mol% (Urzi et al., 2000; Dastager et al., 2008; Kim et al., 2015b). At the time of writing, there are eight recognized species of the genus Marmoricola (http://www.bacterio.net): Marmoricola aquaticus (de Menezes et al., 2015), M. solisilvae and M. terrae (Kim et al., 2015b), M. korecus (Lee et al., 2011), M. scoriae (Lee & Lee, 2010), M. aurantiacus (Urzi et al., 2000), M. aequoreus (Lee, 2007) and M. bigeumensis (Dastager et al., 2008).

In this study, we report on the taxonomic characterization of two strains, designated Gsoil 097T and Gsoil 818T,
which appeared to be members of the genus *Marmoricola*. They were isolated from soil of a ginseng field in Pocheon Province, South Korea. Strains Gsoil 097<sup>T</sup> and Gsoil 818<sup>T</sup> were routinely cultured on R2A agar plates at 30 °C and preserved as a suspension in R2A broth with 20 % (w/v) glycerol at −70 °C.

Extraction of genomic DNA was performed 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 Kim *et al.* (2015a). 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 EzTaxon-e [http://www.ezbiolab.com/eztaxon; Kim *et al.* (2012)]. Multiple alignments were performed by 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 (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods using the MEGA6 program (Tamura *et al.*, 2013) with bootstrap values based on 1000 replications (Felsenstein, 1985).

Nearly complete 16S rRNA gene sequences of strains Gsoil 097<sup>T</sup> (1454 nt) and Gsoil 818<sup>T</sup> (1457 nt) were determined and subjected to comparative analysis. Phylogenetic analysis using the neighbour-joining method based on 16S rRNA gene sequences indicated that strains Gsoil 097<sup>T</sup> and Gsoil 818<sup>T</sup> were clustered within the genus *Marmoricola*, which was also supported by maximum-parsimony and maximum-likelihood trees (Fig. 1). The highest degrees of sequence similarity determined for strains Gsoil 097<sup>T</sup> and Gsoil 818<sup>T</sup> were to *M. solisilvae* KIS18-7<sup>T</sup> (99.1 and 98.3 %, respectively), *M. terrae* JOSS-1<sup>T</sup> (97.9 and 97.9 %), *M. scoriae* SCO-D01<sup>T</sup> (97.8 and 97.1 %), *M. aequoreus* SST-45<sup>T</sup> (97.5 and 97.0 %), *M. aurantiacus* BC 361<sup>T</sup> (97.0 and 96.6 %) and *M. bigeumensis* MSL-05<sup>T</sup> (96.8 and 97.9 %). The two novel strains showed 98.0 % 16S rRNA gene sequence similarity between each other. All species of the genus *Marmoricola* displayed a unique panel of 16S rRNA gene nucleotide patterns that differentiates this genus from the closely related genus *Nocardiodoides* (Fig. S1, available in the online Supplementary Material).

On the basis of these phylogenetic results, *M. solisilvae* KIS18-7<sup>T</sup> (=KACC 17307<sup>T</sup>), *M. terrae* JOSS-1<sup>T</sup> (=KACC 17308<sup>T</sup>), *M. scoriae* SCO-D01<sup>T</sup> (=KCTC 19597<sup>T</sup>), *M. aequoreus* SST-45<sup>T</sup> (=KACC 17353<sup>T</sup>), *M. aurantiacus* BC 361<sup>T</sup> (=KACC 15215<sup>T</sup>) and *M. bigeumensis* MSL-05<sup>T</sup> (=KACC 20988<sup>T</sup>) were selected as the closest recognized neighbours of strains Gsoil 097<sup>T</sup> and Gsoil 818<sup>T</sup> and were obtained from culture collections, grown under the same conditions and used as reference strains in most of the subsequent DNA–DNA hybridization and phenotypic tests.

DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and micro-dilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the remaining three values were used to calculate similarity values. The DNA hybridization values quoted are the means of these three values.

Strain Gsoil 097<sup>T</sup> showed a DNA–DNA relatedness value of 15 ± 2 % with Gsoil 818<sup>T</sup>, 30 ± 4 % with *M. solisilvae* KACC 17307<sup>T</sup>, 11 ± 2 % with *M. terrae* KACC 17308<sup>T</sup>, 7 ± 2 % with *M. bigeumensis* KACC 20988<sup>T</sup>, 10 ± 2 % with *M. scoriae* KCTC 19597<sup>T</sup> and 8 ± 2 % with *M. aequoreus* KACC 17353<sup>T</sup> while strain Gsoil 818<sup>T</sup> showed a DNA–DNA relatedness value of 18 ± 3 % with Gsoil 097<sup>T</sup>, 18 ± 2 % with *M. solisilvae* KACC 17307<sup>T</sup>, 9 ± 2 % with *M. terrae* KACC 17308<sup>T</sup>, 5 ± 2 % with *M. scoriae* KCTC 19597<sup>T</sup> and 3 ± 1 % with *M. aequoreus* KACC 17353<sup>T</sup>. These DNA–DNA relatedness values among the two isolated strains and five closely related type strains of the genus *Marmoricola* are presented in Table S1. According to Wayne *et al.* (1987), DNA–DNA relatedness values lower than 70 % are considered to be the threshold for the delineation of genospecies, so the results obtained are low enough to assign strains Gsoil 097<sup>T</sup> and Gsoil 818<sup>T</sup> to novel species of the genus *Marmoricola*.

The Gram reaction was determined using the non-staining method, as described by Buck (1982). Cell morphology was examined by scanning electron microscopy (Hitachi SU-3500), using cells grown for 3 days at 30 °C on R2A agar. Cell motility was determined using the hanging drop method. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman (2002). Biochemical tests were carried out by using API 20NE, API ID 32GN and API ZYM kits according to the instructions of the manufacturer (bioMérieux). Tests for degradation of DNA (using DNase agar from Scharlau, with DNase activity assessed by flooding plates with 1 M HCl), casein, starch (Atlas, 1993), xylan and CM-cellulose (Ten *et al.*, 2004) were performed and evaluated after 7 days. Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 40, 42 and 50 °C) and various pH values (pH 3.5–10.0 at intervals of 0.5 pH units) was assessed after 7 days of incubation at 30 °C. Three different 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 R2A medium supplemented with 1–10 % (w/v, at intervals of 1 %) NaCl and growth was assessed after 7 days of incubation. Growth on nutrient agar (NA; BD), trypticase soy agar (TSA; BD) and MacConkey agar (BD) was also evaluated at 30 °C.

Cells of both strain Gsoil 097<sup>T</sup> and strain Gsoil 818<sup>T</sup> were Gram-reaction-positive, aerobic, non-motile, non-spor-forming and rod-shaped (Fig. S2). Colonies of both strains grown on R2A agar for 3 days were circular, convex, translucent, ivory-coloured and 0.5–1 mm in diameter. Neither strain grew on TSA or MacConkey
agar, whereas both strains grew weakly on NA at 30 °C. Physiological and biochemical characteristics of strains Gsoil 097T and Gsoil 818T are summarized in the species descriptions and a comparison of selective characteristics of strains Gsoil 097T, Gsoil 818T and related type strains is given in Table 1.

For measurement of the DNA G+C content, the genomic DNA of the novel strains was extracted and purified as described by Moore & Dowhan (1995), and then degraded enzymically into nucleosides. The G+C content was determined as described by Mesbah et al. (1989) using reversed-phase HPLC. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), evaporated under vacuum conditions, and re-extracted 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 analysed by HPLC as previously described (Hiraishi et al., 1996). Cellular fatty acid profiles were determined for strains grown on R2A agar for 3 days 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 acids were analysed by GC (Hewlett Packard 6890) and identified by the Microbial Identification software package based on the Sherlock Aerobic Bacterial Database (TSBA60) (Sasser, 1990). The isomer of the diamino acid of the cell-wall peptidoglycan was determined by using TLC, after hydrolysis of whole cells with 6 M HCl at 100 °C for 18 h, as described by Komagata & Suzuki (1988). Polar lipids were extracted and examined by two-dimensional TLC (Minnikin et al., 1984).

The DNA G+C contents of strains Gsoil 097T and Gsoil 818T were 68.8 and 70.0 mol%, respectively, which were similar to those of recognized species of the genus Marmoricola except M. terrae JOS5-1T which has a relatively low G+C content (Table 1). The major respiratory quinone

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**Fig. 1.** Phylogenetic relationship of strains Gsoil 097T and Gsoil 818T with recognized species of the genus Marmoricola and other related species. The tree was reconstructed by using the neighbour-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-parsimony and maximum-likelihood methods. Bar, 0.005 substitutions per nucleotide position.
for both strain Gsoil 097T and strain Gsoil 818T was MK-8(H₄). A small quantity of MK-7(H₄) was also detected at a peak area ratio of less than 3 % for both strains. L-L-Diaminopimelic acid was detected in the whole-cell hydrolysates. The fatty acid profiles of strains Gsoil 097T and Gsoil 818T were compared with those of the type strains of recognized *Marmoricola* species. The major fatty acids of strain Gsoil 097T were C₁₇:0 10-methyl (11.7 %), C₁₈:0 9c (11.1 %), C₁₈:0 10-methyl (17.8 %), iso-C₁₇:0 3 (13.4 %), C₁₆:0 10-methyl (11.6 %), C₁₇:1 9c (10.7 %), and iso-C₁₆:0 10c (10.6 %), and these components were also present in *Marmoricola* species. However, some qualitative and quantitative differences in the fatty acids distinguished strains Gsoil 097T and Gsoil 818T from each other and

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**Table 1. Phenotypic characteristics of strains Gsoil 097T and Gsoil 818T and the type strains of related *Marmoricola* species**

<table>
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Reference data were taken from: a, Lee (2007); b, Urzi et al. (2000); c, Dastager et al. (2008); d, Lee & Lee (2010); e, Kim et al. (2015b).
from the other recognized species of the genus *Marmoricola* (Table S2). The two strains had the same polar lipid profile: diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, an unidentified aminolipid and unidentified polar lipids (Fig. S3).

On the basis of the data and observations described above, it is appropriate to conclude that Gsoil 097T and Gsoil 818T should be assigned to the genus *Marmoricola* as the type strains of two novel species, for which the names *Marmoricola ginsengisoli* sp. nov. and *Marmoricola pocheonensis* sp. nov. are proposed, respectively.

**Description of *Marmoricola ginsengisoli* sp. nov.**

*Marmoricola ginsengisoli* (gin.sen.gi.so’li. N.L. n. ginsengum ginseng; L. n. solum soil; N.L. gen. n. ginsengisoli of soil of a ginseng field, the source of the organism).

Cells are Gram-reaction-positive, aerobic, non-motile, non-spore-forming and rod-shaped (0.3–0.4 μm in diameter and 0.8–1.4 μm in length). Colonies grown on R2A agar plates for 3 days are circular, convex, translucent, ivory-coloured and 0.5–1 mm in diameter. Catalase-positive and oxidase-positive. Growth occurs at 10–37 °C and at pH 6.0–8.0. Optimum growth occurs at 30 °C and at pH 7.0 without additional NaCl supplement. Growth is inhibited in the presence of 2.0 % (w/v) NaCl. Hydrolysates skimmed milk but not starch, cellulose or DNA. Nitrate is reduced to nitrite. According to API 20NE and API ID 32GN test strips, positive for aesculin hydrolysis but negative for gelatin hydrolysis. Assimilates glucose, adipect, D-glucose, L-arabinose, valerate, L-histidine, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, N-acetyl-D-glucosamine, inositol and glycogen, but not D-mannose, maltose, malate, phenyl-acetate, D-mannitol, salicin, melibiose, L-fucose, D-sorbitol, propionate, 2-keto-glucuronate, D-ribose, caprate, citrate, succrose, itaconate, suberate, malonate, acetate, lactate, L-alanine, 5-ketogluconate, 3-hydroxy-benzoate or L-serine. Enzyme activities (API ZYM) are listed in Table 1. Major fatty acids are C18 : 1ω7c, C18 : 0, 10-methyl, iso-C17 : 0, C16 : 0, 10-methyl, C17 : 1ω9c, C18 : 1ω9c and iso-C16 : 0. Polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, an unidentified aminolipid and unidentified polar lipids. The peptidoglycan contains LL-diaminopimelic acid as the diagnostic diamino acid. The major menaquinon is MK-8(H4).

The type strain is Gsoil 818T (= KACC 14275T, DSM 22773T), isolated from a ginseng field in Pocheon Province, South Korea. The DNA G+C content of the type strain is 70.0 mol%.

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**References**


Two novel Marmoricola species


