# *Humibacter ginsengiterrae* sp. nov., and *Humibacter ginsengisoli* sp. nov., isolated from soil of a ginseng field

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Two novel Gram-staining-positive bacteria, designated DCY60$^T$ and DCY90$^T$, were isolated from soil of a ginseng field in the Republic of Korea. 16S rRNA gene sequence comparisons showed the two novel strains were closely related to members of the genus *Humibacter* with greatest similarity to *Humibacter antri* KCTC 33009$^T$ (98.8 and 98.4 % for DCY60$^T$ and DCY90$^T$, respectively). The predominant menaquinones present were MK-11 and MK-12. The major fatty acids were anteiso-$C_{17:0}$ and summed feature 8 containing $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$. The DNA G+C contents of strains DCY60$^T$ and DCY90$^T$ were 62.8 and 66.8 mol%, respectively. The peptidoglycan of both strains contained the amino acids ornithine, 2,4-diaminobutyric acid, alanine, glutamic acid and glycine. The cell-wall sugars of strain DCY60$^T$ comprised glucose, galactose, rhamnose and xylose, while strain DCY90$^T$ contained glucose, galactose, rhamnose and ribose. The major polar lipids of both strains were phosphatidylglycerol, an unidentified glycolipid, and an unknown phospholipid. On the basis of the phenotypic analysis strains DCY60$^T$ and DCY90$^T$ represent novel species of the genus *Humibacter,* for which names *Humibacter ginsengiterrae* sp. nov. (type strain DCY60$^T$=KCTC 33520$^T$=JCM 30079$^T$) and *Humibacter ginsengisoli* sp. nov. (type strain DCY90$^T$=KCTC 33521$^T$=JCM 30080$^T$) are proposed.

Ginseng (*Panax ginseng* C.A. Meyer) is mainly cultivated in China, Korea and Canada. It has been regarded as one of the most important remedies in oriental medicine for more than 1000 years (Yu *et al.*, 2003). The ginseng plant grows best under conditions that simulate its natural habitat. It requires 70–90 % artificial or natural shade. Ginseng thrives in a climate with 4–10 cm of annual precipitation and a temperature from 16–18 °C (Yang, 1974). It requires several weeks of cold temperature for adequate dormancy. Ginseng generally prefers a deep (20–30 cm), well-drained, loamy soil with a high organic content and a pH near 5.5 (Hong, 1978). Very sandy soils tend to produce plants with long, slender roots of inferior quality. Ginseng requires 3 to 5 years to produce a marketable crop. Many novel bacterial species have been found in ginseng soil, belonging to four phyla *Proteobacteria*, Bacteroidetes, *Firmicutes*, and Actinobacteria. In this study, we have characterized two ginseng soil isolates, strains DCY60$^T$ and DCY90$^T$ with phenotypic, chemotaxonomic and phylogenetic analyses and found the affiliation of these isolates to the genus *Humibacter*. Strains DCY60$^T$ and DCY90$^T$ are classified as representing two distinct novel species based on the evidence presented below.

The genus *Humibacter* was first established by Vaz-Moreira *et al.* (2008) with *Humibacter albus* as the type species. At the time of writing, the genus *Humibacter* includes two recognized species (http://www.bacterio.net/humibacter.html), the type species and *Humibacter antri* (Lee, 2013), isolated from a cave clay soil sample. Species of the genus *Humibacter* are Gram-stain-positive, motile or non-motile, with short rod-like shape. Members of the

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Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Humibacter ginsengiterrae* sp. nov. DCY60$^T$ and *Humibacter ginsengisoli* sp. nov. DCY90$^T$ are JQ010859 and KF915800, respectively.

Three supplementary figures are available with the online Supplementary Material.

genus have ornithine and 2,4-diaminbutyric acid as diagnostic diamino acids in their cell-wall peptidoglycan with *N*-acetylated murein, while the major menaquinones are MK-11 and MK-12. The major fatty acids are cyclohexyl-$C_{17:0}$, anteiso-$C_{17:0}$, iso-$C_{16:0}$ and anteiso-$C_{15:0}$. The polar lipids comprise phosphatidylglycerol, glycolipid and phospholipid.

Strains DCY60$^T$ and DCY90$^T$ were isolated from ginseng soil samples (sample Y1 obtained from Yeoncheon County and sample G1 from Gochang County, Republic of Korea) by plating of serial dilutions up to $10^{-4}$ on R2A agar (Difco). Single colonies were selected and transferred onto new plates for purification. Routine cultivation was performed on R2A agar at 30 °C and the isolates were stored at −80 °C in R2A broth (Difco) supplemented with 25 % (v/v) glycerol. For further testing, we used trypticase soy agar (TSA; Difco) for optimal growth of both strains.

Colony morphology was observed after cultivation on TSA for 4 days at 30 °C. Cell size, shape, morphology and flagellation of strains DCY60$^T$ and DCY90$^T$ were observed by phase-contrast microscopy (×1000 magnification, Nikon Optiphot-2) and by transmission electron microscopy after growth on TSA for 1 day at 30 °C. Motility was determined by the hanging-drop technique (Bernardet *et al.*, 2002). Gram staining was performed using a bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase activity was analysed by the measurement of bubble production after application of 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined by using 1 % (w/v) *N,N,N,N*-tetramethyl-1,4-phenylenediamine reagent (bioMérieux) according to the manufacturer's instructions. Growth of the strains was assessed on different media: nutrient agar (NA; Difco), TSA, R2A, MacConkey (Difco) and Luria–Bertani (LB; Difco) at 30 °C. The temperature range for growth was tested by checking growth in trypticase soy broth (TSB) and on TSA at 4, 10, 15, 25, 30, 37 and 40 °C. Tolerance for salinity was evaluated in TSB supplemented with [0–10.0 % (w/v) NaCl, at 1.0 % intervals] at 30 °C. The pH range for growth was examined from pH 3.0–10.0 using 1.0 pH unit intervals in TSB adjusted with 10 mM phosphate/citrate buffer (for pH 4.0–5.0), MES buffer (pH 6.0), HEPES buffer (pH 7.0–8.0), Tris buffer (pH 9.0) and $NaHCO_3/Na_2CO_3$ (pH 10.0). For pH values from 3.0, TSB was adjusted by the addition of 1 M HCl after sterilization. $H_2S$ production was evaluated on triple-sugar iron agar. Indole production was analysed using Kovács' reagent in 1 % tryptone broth. Nitrate reduction was tested in nitrate broth containing 0.2 % $KNO_3$ (Skerman, 1967). Urease activity was evaluated in Christensen's medium (Christensen, 1946). DNase activity, hydrolysis of casein, gelatin and aesculin were also checked by the standard described methods (Prescott & Harley, 2001). Carbon utilization and enzyme production were examined using the API 20NE, API 50CH and API ZYM strips (bioMérieux) according to the manufacturer's instructions. The results of API 50CH and API 20NE strips were recorded after 24 h and those of API ZYM strips were recorded after incubation for 6 h. Antibiotic susceptibilities were checked according to the Kirby–Bauer method (Bauer *et al.*, 1966). The following antibiotics were tested: tetracycline (30 μg), neomycin (30 μg), erythromycin (15 μg), oleandomycin (5 μg), ceftazidime (30 μg), rifampicin (5 μg), novobiocin (34 μg), carbenicillin (100 μg), penicillin (10 μg), cephazolin (30 μg) (Oxoid). Inhibition zones were interpreted according to the manufacturer's manual.

Strain DCY60$^T$ formed ivory, circular and smooth colonies that were approximately 0.3–1.0 mm in diameter after 4 days cultivation on TSA at 30 °C, while strain DCY90$^T$ formed white–cream, circular, entire and smooth colonies that were approximately 0.5–1.3 mm in diameter after 4 days cultivation on TSA at 30 °C. Both strains were oxidase-negative, catalase-positive, Gram-reaction-positive, non-motile, non-flagellated, rod-shaped, and approximately $0.5 \times 1.3$ μm for strain DCY60$^T$ and approximately $0.4 \times 0.8$ μm for strain DCY90$^T$ (Fig. S1, available in the online Supplementary Material). Both strains grew at 15–37 °C (optimum 30 °C). Strain DCY60$^T$ exhibited growth with NaCl concentrations of 0–5.0 %; no growth was observed with 6 % NaCl. Strain DCY90$^T$ exhibited growth with NaCl concentrations of 0.0–4.0 %; no growth was observed with 5 % NaCl. The pH range for growth of the strains was pH 4.0–8.0 (optimum pH 5.0–6.0). Both strains grew on NA, TSA, R2A and LB but not on MacConkey agar at 30 °C. Both strains could not hydrolyse Tweens 20 and 80, casein, starch, cellulose and gelatin. Strain DCY90$^T$ hydrolysed DNA, but strain DCY60$^T$ did not. The results of other physiological and biochemical analyses are summarized in Table 1 and the species descriptions.

Genomic DNA of strains DCY60$^T$ and DCY90$^T$ were extracted and purification by using Wizard Genomic DNA purification kit (Promega) according to the manufacturer's instructions. The 16S rRNA gene was amplified with universal bacterial primers 27F, 518F, 800R and 1492R (Lane, 1991; Anzai *et al.*, 2000). The purified PCR products were sequenced by Genotech (Daejeon, Republic of Korea). Comparison of 16S rRNA gene sequences from the novel strains with existing sequences from the published database was carried out using the EzTaxon-e server (http://www.ezbiocloud.net/eztaxon) (Kim *et al.*, 2012). Further, the almost-complete 16S rRNA gene sequences of the novel strains and closely related strains were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997) and Kimura's two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed by the neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-parsimony method (MP) (Fitch, 1971) and maximum-likelihood (ML) algorithms by using the MEGA 5 software package (Tamura *et al.*, 2011). A total of 1360 nt present in all strains was used for tree reconstructions. Bootstrap analysis based on 1000 replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). The DNA G+C content was

**Table 1.** Differential characteristics of strains DCY60$^T$ and DCY90$^T$ and type strains of other related species of the genus *Humibacter*

Strains: 1, *Humibacter ginsengiterrae* sp. nov. DCY60$^T$; 2, *Humibacter ginsengisoli* sp. nov. DCY90$^T$; 3, *H. antri* KCTC 33009$^T$; 4, *H. albus* LMG 23996$^T$. All strains are positive for Gram-staining, nitrate reduction to nitrites, and activity of esterase (C4), lipase (C8), leucine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. All strains are negative for lipase (C14), trypsin, α-chymotrypsin, gelatinase, arginine dihydrolase, indole production and glucose fermentation. All strains assimilated D-glucose and *N*-acetylglucosamine, but not potassium gluconate, capric acid or L-arabinose (API ZYM and API 20NE). All strains produced acid from D-glucose, D-mannose, *N*-acetylglucosamine and cellobiose, but not from glycerol, L-xylose, D-adonitol, L-sorbose, dulcitol, D-sorbitol, inulin, melezitose, starch, glycogen, D-tagatose, L-arabitol, potassium 2-ketogluconate, potassium 5-ketogluconate (API 50CH). +, Positive; −, negative.

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Urease | − | − | − | + |
| Hydrolysis of aesculin | + | − | + | + |
| Enzyme activity (API ZYM) | | | | |
| Alkaline phosphatase | + | + | − | − |
| Valine arylamidase | + | + | − | + |
| Cystine arylamidase | + | + | + | − |
| Naphthol-AS-BI-phosphohydrolase | + | + | − | − |
| β-Glucuronidase | + | + | − | − |
| Assimilation of (API 20NE): | | | | |
| D-Mannose | + | + | + | − |
| D-Mannitol | + | − | − | + |
| Maltose | − | − | + | − |
| Adipic acid | + | + | − | − |
| Malate | − | + | + | + |
| Trisodium citrate | + | − | − | + |
| Phenylacetic acid | + | − | − | + |
| Acid production from (API 50CH): | | | | |
| Erythritol | − | + | − | − |
| D-Arabinose | − | + | + | + |
| L-Arabinose | − | + | − | + |
| D-Ribose | − | + | − | + |
| D-Xylose | + | + | − | + |
| Methyl β-D-xylopyranoside | + | + | − | + |
| D-Galactose | − | + | − | + |
| D-Fructose | + | + | − | + |
| L-Rhamnose | + | + | − | + |
| Inositol | + | + | − | − |
| D-Mannitol | − | − | − | + |
| Methyl α-D-mannopyranoside | − | − | + | − |
| Methyl α-D-glucopyranoside | − | + | + | − |
| Amygdalin | − | − | − | + |
| Arbutin | + | − | + | + |
| Aesculin | + | − | + | + |
| Salicin | + | − | + | + |
| Lactose | − | − | − | + |
| Melibiose | − | − | − | + |
| Sucrose | − | + | + | + |
| Trehalose | − | + | − | + |
| Raffinose | − | + | − | − |
| Xylitol | − | − | + | + |
| Gentiobiose | − | + | − | + |
| Turanose | − | + | − | − |
| D-Lyxose | − | + | − | − |
| D-Fucose | − | − | + | + |
| L-Fucose | + | + | − | − |
| D-Arabitol | − | − | − | + |

determined by the method of Mesbah *et al.* (1989). The obtained nucleoside mixture was separated by HPLC [NS-6000A, Futecs; reversed-phase column YMC-Triart C18 (4.6 × 250 mm × 5 μm). The mobile phase used was 20 mM $NH_4H_2PO_4$/acetonitrile (20 : 1; v/v)]. Genomic DNA from *Escherichia coli* strain B (D4889; Sigma-Aldrich) was used as a standard. DNA–DNA hybridization was performed fluorometriccally, according to the method of Ezaki *et al.* (1989), using photobiotin-labelled DNA probes and microdilution wells with optimal hybridization temperatures of strain DCY60$^T$ and DCY90$^T$, which were 50.1 and 51.7 °C, respectively. The hybridization was conducted in five replications for each sample. The highest and lowest values obtained for each sample were excluded, and the mean of the remaining three values are quoted as DNA–DNA relatedness values.

Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strains DCY60$^T$ and DCY90$^T$ belonged to the genus *Humibacter* (Fig. 1, Fig. S2). Strains DCY60$^T$ and DCY90$^T$ were most closely related to *Humibacter antri* KCTC 33009$^T$, sharing 98.9 % and 98.3 % 16S rRNA gene sequence similarity, respectively. Strains DCY60$^T$ and DCY90$^T$ formed a reliable and monophyletic cluster with *H. antri* KCTC 33009$^T$ in the ML and MP algorithms with high bootstrap values (Fig. 1). The similarity value between strains DCY60$^T$ and DCY90$^T$ was 98.5 %. The DNA G+C contents of strains DCY60$^T$ and DCY90$^T$ were 62.8 and 66.8 mol%, respectively. The DNA–DNA relatedness values between strain DCY60$^T$ and strain DCY90$^T$, *H. albus* LMG 23996$^T$ and *H. antri* KCTC 33009$^T$ were 47.6±0.9, 26.2±0.6 and 15.6±0.9 %, respectively. Meanwhile, strain DCY90$^T$ showed DNA–DNA relatedness values of 47.5±1.0, 32.1±0.7 and 20.0±1.1 % with strain DCY60$^T$, *H. albus* LMG 23996$^T$ and *H. antri* KCTC 33009$^T$, respectively.

For isoprenoid quinone analysis, cell biomass was grown in R2A broth medium (Difco) at 30 °C for 48 h and then freeze-dried. Menaquinones were extracted from freeze-dried cells (50 mg) with chloroform/methanol (2 : 1, v/v), purified using Sep-Pak Vac 6cc silica cartridge (Waters) and subsequently analysed by HPLC as described by Collins (1985). Cellular fatty acids were saponified, methylated and

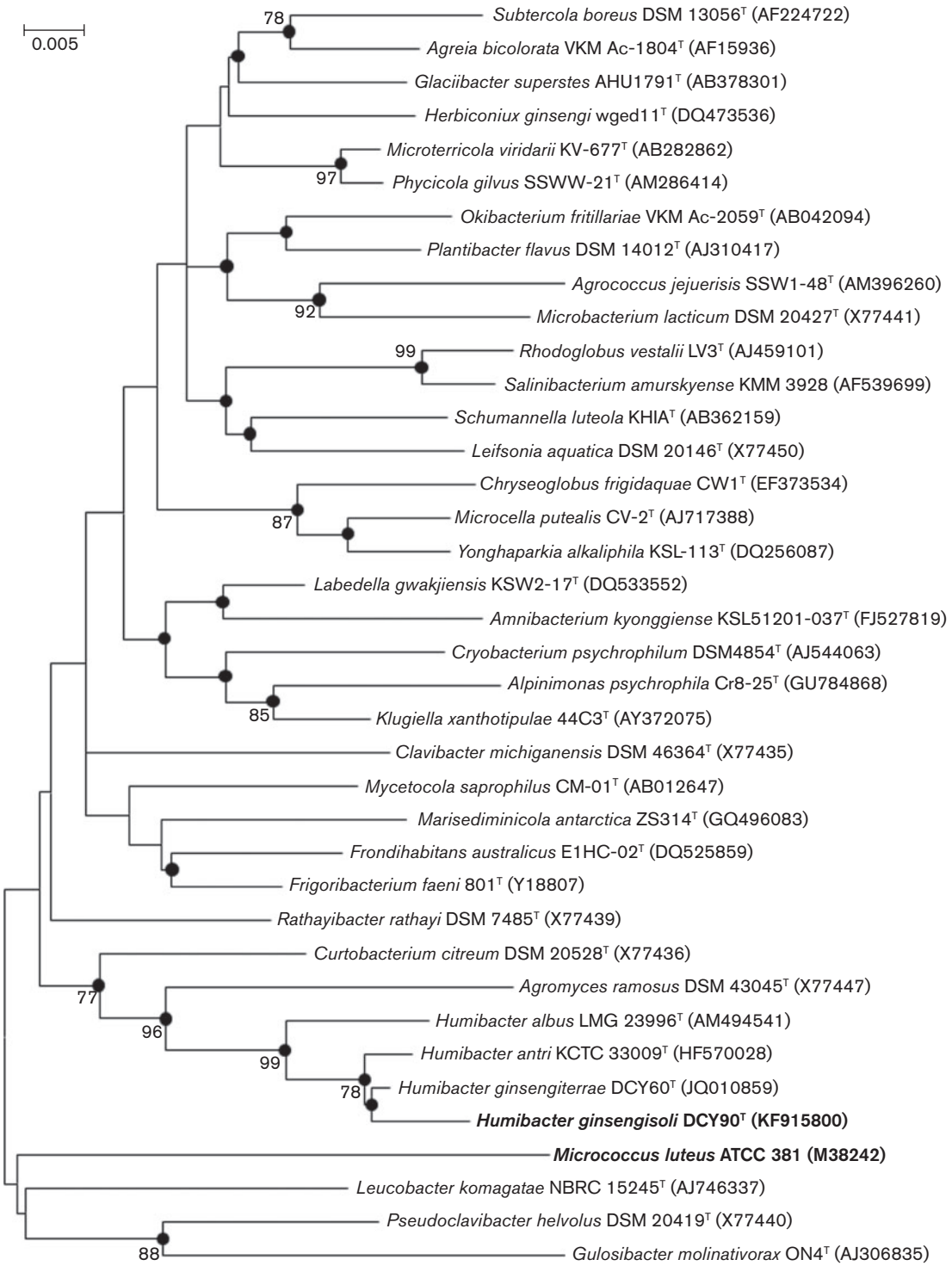


**Fig. 1.** Phylogenetic tree derived from 16S rRNA gene sequences of strains DCY60$^T$ and DCY90$^T$ and their taxonomic neighbours, reconstructed with maximum–likelihood method. Bootstrap values (>70 %) based on 1000 replicates are shown at branch node. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.005 substitutions per nucleotide position.

extracted according to the protocol of the Sherlock Microbial Identification System (Sasser, 1990). The fatty acid methyl esters were analysed by GC (Hewlett Packard 6890) with Sherlock MIDI software (version 6.0) and a TSBA database (version 6.0). The polar lipids of strains DCY60$^T$, DCY90$^T$ and *H. albus* LMG 23996$^T$ were extracted and analysed by

two-dimensional thin layer chromatography. For the presence of all lipids, TLC plates were sprayed with 5 % molybdophosphoric acid followed by charring at 120 °C for 15 min. Amino lipids were detected by spraying with 0.2 % ninhydrin and charring at 110 °C for 10 min. Glycolipids were detected by spraying with 5 % α-naphthol and charring at 110 °C for 10 min. Phospholipids were detected by spraying with molybdenum blue reagent (Minnikin *et al.*, 1984). Cell-wall sugars of strains DCY60$^T$ and DCY90$^T$ were determined by TLC according to Becker *et al.* (1965). For this, 50 mg freeze-dried cells were hydrolysed in 1 ml $H_2SO_4$ (1 M) at 95 °C for 2 h. The pH of the samples was then adjusted to pH 5.0–5.5 with saturated $Ba(OH)_2$. Centrifugation (1500 r.p.m., 5 min) precipitated the resultant $BaSO_4$. The liquid phase was evaporated with a rotary evaporator and the residue was dissolved in 4 ml $H_2O$, and then centrifuged to remove particles. Cell-wall sugar extraction was determined by spotting samples onto TLC cellulose (Merck, 20 × 20 cm). The solvent system used was ethylacetate/pyridine/water (50 : 30 : 20, by vol.). Sugars were detected by spraying with anilinphthalate [0.8 g phthalic acid (Sigma Aldrich, P/N 402915), 2 ml distilled water, 24 ml 1-butanol, 24 ml diethylether and 0.5 ml aniline (Sigma Aldrich, P/N 242284)]. Peptidoglycan of strains DCY60$^T$ and DCY90$^T$ were determined by TLC according to McPherson & Popham (2003). Freeze-dried cells (200 mg) were dissolved in 50 mM phosphate buffer (pH 7.2), and then sonicated three times (10 min) in ice water. Cell suspensions were centrifuged at 4000 ***g*** for 20 min to remove unlysed cells. The supernatants were collected in new tubes and centrifuged again at 40 000 ***g*** for 25 min. The supernatants were discarded and pellets which contain the peptidoglycan were collected. The residues were suspended with 4 % SDS solution (6 ml) in a glass tube and heated at 100 °C in an oven until it became colourless. The samples were then centrifuged again at 40 000 ***g*** for 25 min at room temperature. The collected pellets were washed three times with distilled water and centrifuged at 40 000 ***g*** for 25 min at room temperature. The pellet was dried at 50 °C and then 5 mg material was hydrolysed in 6 M HCl at 100 °C for 16 h. The hydrolysed peptidoglycans were centrifuged at 13 000 ***g*** for 5 min and the samples were then filtered through a 0.2 μm-filter. HCl was removed using a vacuum evaporator, and dried samples were finally dissolved in 0.3 ml water. The chemical content of the hydrolysed peptidoglycan was determined by spotting the sample on TLC cellulose Merck KGaA plates (20 × 20 cm). The solvent system used was methanol/pyridine/HCl/water (100 : 12.5 : 6 : 32.5, by vol.).

The predominant isoprenoid quinones of strain DCY60$^T$ were MK-11 (79.1 %) and MK-12 (20.9 %), while for strain DCY90$^T$ were MK-11 (74.2 %), MK-12 (17.1 %) and MK-10 (8.7 %). The major polar lipids of the novel isolates consisted of diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. Both DCY60$^T$ and DCY90$^T$ showed polar lipid profiles similar to that of *H. albus* LMG 23996$^T$. An unidentified aminoglycolipid (AGL) was found in strain DCY90$^T$ that was not found in strain DCY60$^T$ or *H. albus* LMG 23996$^T$ (Fig. S3). The major cellular fatty acids (>10 %) of strain DCY60$^T$ were anteiso-$C_{17:0}$, summed feature 8 (containing $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and iso-$C_{16:0}$, while those of strain DCY90$^T$ were summed feature 8 (containing $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and anteiso-$C_{17:0}$. The fatty acid profiles of the two strains showed similarity with the type strains of species of the genus *Humibacter* (Table 2). The peptidoglycan of strains DCY60$^T$ and DCY90$^T$ contained the amino acids ornithine, 2.4-diaminobutyric acid, alanine, glutamic acid and glycine. This profile was similar to that of described species of the genus *Humibacter*. The cell-wall sugars of strain DCY60$^T$ contained glucose, galactose, rhamnose and xylose, while strain DCY90$^T$ contained glucose, galactose, rhamnose and ribose.

The results of the phylogenetic and chemotaxonomic analyses suggested the two isolated strains belong to the genus *Humibacter*. The DNA–DNA relatedness among strains DCY60$^T$, DCY90$^T$ and related species of the genus *Humibacter* were lower than 50 %; these values are well-below the 70 % threshold proposed for species delineation (Wayne *et al.*, 1987). The results of physiological and biochemical tests enabled strains DCY60$^T$ and DCY90$^T$ to be distinguished from other members of genus *Humibacter*. Therefore, it proposed that strains DCY60$^T$ and DCY90$^T$ be classified as representatives of two novel species of the genus *Humibacter*, for which the names *Humibacter ginsengiterrae* sp. nov. and *Humibacter ginsengisoli* sp. nov., respectively, are proposed.

**Table 2.** Cellular fatty acid profiles of strains DCY60$^T$ and DCY90$^T$ and type strains of other related species of the genus *Humibacter*

Strains: 1, *Humibacter ginsengiterrae* sp. nov. DCY60$^T$; 2, *Humibacter ginsengisoli* sp. nov. DCY90$^T$; 3, *H. antri* KCTC 33009$^T$; 4, *H. albus* LMG 23996$^T$. All type strains were collected after 24 h growth on TSA medium (Difco) at 30 °C. All data are from this study. Fatty acids <0.5 % are not listed. TR, Traces (<0.5 %).

| Fatty acid | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| iso-$C_{15:0}$ | 3.3 | 2.9 | 4.4 | 2.8 |
| anteiso-$C_{15:0}$ | 7.4 | 3.8 | 6.5 | 8.2 |
| iso-$C_{16:0}$ | 10.2 | 7.4 | 10.5 | 18.3 |
| $C_{16:0}$ | TR | 0.6 | TR | 0.8 |
| iso-$C_{17:0}$ | 3.8 | 4.8 | 4.8 | 3.7 |
| anteiso-$C_{17:0}$ | 42.6 | 30.4 | 44.4 | 34.6 |
| Summed feature 8* | 31.8 | 49.3 | 27.9 | 30.3 |

*Summed features are groups of two or more fatty acids that could not be separated by GLC with the Microbial Identification System (MIDI). Summed feature 8 contained $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$.

### Description of *Humibacter ginsengiterrae* sp. nov.

*Humibacter ginsengiterrae* (gin.sen.gi.ter′rae. N.L. n. *ginsengum* ginseng; L. n. *terra -ae* soil; N.L. gen. n. *ginsengiterrae* from soil of a ginseng field).

Colonies are ivory, circular, smooth and approximately 0.3–1.0 mm in diameter after 4 days cultivation on TSA agar medium at 30 °C. Cells are oxidase-negative, catalase-positive, Gram-reaction-positive, non-motile, non-flagellated, rod-shaped and approximately 0.5 × 1.3 μm. Grows at 15–37 °C (optimum 30 °C), and with NaCl concentration of 0–5.0 %; no growth is observed with 6 % NaCl or higher. The pH range for growth is pH 4.0–8.0 (optimum pH 5.0–6.0).Growth occurs on NA, TSA, R2A and LB agars, but not on MacConkey agar at 30 °C. Does not hydrolyse Tweens 20 and 80, casein, starch, cellulose, gelatin or DNA. According to the API ZYM test, positive for esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, alkaline phosphatase and β-glucuronidase activities, but negative for lipase (C14), trypsin and α-chymotrypsin activities. According to the API 20NE test, positive for nitrate reduction to nitrites, aesculin hydrolysis, β-galactosidase and assimilation of D-glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, adipic acid, trisodium citrate and phenylacetic acid; negative for glucose fermentation, gelatinase, arginine dihydrolase, urease, indole production, and assimilation of L-arabinose, maltose, potassium gluconate, capric acid and malate. According to the API 50 CH test, positive for acid production from D-xylose, methyl β-D-xylopyranoside, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, *N*-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose and L-fucose, but negative for acid production from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, L-xylose, D-adonitol, D-galactose, L-sorbose, dulcitol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-arabitol, D-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The predominant menaquinones are MK-11 and MK-12. The cell-wall peptidoglycan contains the amino acids ornithine, 2.4-diaminobutyric acid, alanine, glutamic acid and glycine. The cell-wall sugars contain glucose, galactose, rhamnose and xylose. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The major cellular fatty acids (> 10 %) are anteiso-$C_{17:0}$, summed feature 8 (containing $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and iso-$C_{16:0}$.

The type strain is DCY60$^T$ (=KCTC 33520$^T$=JCM 30079$^T$), isolated from soil of a ginseng field in Yeoncheon county (38° 04′ 00″ N 126° 57′ 00″ E), Republic of Korea. The DNA G+C content of the type strain is 62.8 mol%.

### Description of *Humibacter ginsengisoli* sp. nov.

*Humibacter 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).

Colonies are white–cream, circular, smooth and approximately 0.5–1.3 mm in diameter after 4 days cultivation at 30 °C. Cells are oxidase-negative, catalase-positive, Gram-reaction-positive, non-motile, non-flagellated, rod-shaped and approximately 0.4 × 0.8 μm. Grows at 15–37 °C (optimum 30 °C), at pH 4.0–8.0 (optimum pH 5.0–6.0) and with NaCl concentrations of 0–4.0 %. Grows on NA, TSA, R2A and LB agars, but not on MacConkey agar at 30 °C. DNA is hydrolysed, but Tweens 20 and 80, casein, starch, cellulose and gelatin are not. According to the API ZYM test, positive for esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, alkaline phosphatase and β-glucuronidase activities, but negative for lipase (C14), trypsin and α-chymotrypsin activities. According to the API 20NE test, positive for β-galactosidase, nitrate reduction to nitrites, and assimilation of D-glucose, malate, adipic acid, *N*-acetylglucosamine and D-mannose; negative for gelatinase, indole production, urease, aesculin hydrolysis, arginine dihydrolase, glucose fermentation, and assimilation of trisodium citrate, phenylacetic acid, maltose, potassium gluconate, capric acid, L-arabinose and D-mannitol. According to the API 50CH test, positive for acid production from erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, inositol, methyl α-D-glucopyranoside, *N*-acetylglucosamine, cellobiose, maltose, sucrose, trehalose, raffinose, gentiobiose, turanose, D-lyxose and D-fucose; negative for acid production from glycerol, L-xylose, D-adonitol, L-sorbose, dulcitol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, amygdalin, arbutin, salicin, lactose, melibiose, inulin, melezitose, starch, glycogen, aesculin, xylitol, D-tagatose, L-fucose, L-arabitol, D-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. The predominant menaquinones are MK-10, MK-11 and MK-12. The cell-wall peptidoglycan contains the amino acids ornithine, 2.4-diaminobutyric acid, alanine, glutamic acid and glycine; the cell-wall sugars contain glucose, galactose, rhamnose and ribose. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The major cellular fatty acids are summed feature 8 (containing $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$) and anteiso-$C_{17:0}$.

The type strain is DCY90$^T$ (=KCTC 33521$^T$=JCM 30080$^T$) isolated from soil of a ginseng field in Gochang county (35° 26′ 89″ N 126° 42′ 74″ E), Republic of Korea. The DNA G+C content of the type strain is 66.8 mol%.

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