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NOTE

Panacagrimonas perspica gen. nov., sp. nov., a Novel Member of *Gammaproteobacteria* Isolated from Soil of a Ginseng Field§

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A taxonomic study was carried out on Gsoil 142^T, a bacterial strain isolated from the soil collected in a ginseng field in Pocheon province, South Korea. Comparative 16S rRNA gene sequence studies showed a clear affiliation of this bacterium to the *Gammaproteobacteria*, and it was most closely related to *Hydrocarboniphaga effusa* ATCC BAA 332^T (94.4%, 16S rRNA gene sequence similarity), *Nevskia ramosa* DSM 11499^T (94.1%) and *Alkanibacter difficilis* MN154.3^T (92.0%). Strain Gsoil 142^T was a Gram-negative, strictly aerobic, motile, and rod-shaped bacterium. The G+C content of the genomic DNA was 69.9% and predominant ubiquinone was Q-8. Major fatty acids were summed feature 8 ($C_{18:1}$ *ω7c* and/or *ω6c*, 36.3%), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ *ω7c*, 20.6%) and $C_{16:0}$ (17.4%). The major polar lipids detected in strain Gsoil 142^T were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, and an unknown glycolipid. On the basis of polyphasic evidence, it is proposed that strain Gsoil 142^T should be placed in a novel genus and species, for which the name *Panacagrimonas perspica* gen. nov., sp. nov. is proposed. The type strain is Gsoil 142^T (=KCTC 12982^T =LMG 23239^T).

***Keywords*:** 16S rRNA gene, polyphasic taxonomy, *Panacagrimonas perspica* gen. nov., sp. nov.

During a course of study on the culturable aerobic and facultative anaerobic bacterial community of ginseng field soil in Pocheon province (South Korea), a large number of bacteria were isolated (Im *et al*., 2005). One of these isolates, strain Gsoil 142^T, was identified as belonging to the *Gammaproteobacteria* and was the subject of further taxonomic investigation. Here, its taxonomic position, including the genotypic, chemotaxonomic and classical phenotypic characteristics, is reported in order to establish its phylogenetic affiliation. The findings allow the proposal that the strain Gsoil 142^T should be placed in a novel genus and species. The proposed name for this is *Panacagrimonas perspica* gen. nov., sp. nov.

The strain Gsoil 142^T was originally isolated from soil in a ginseng field in Pocheon province in South Korea. This soil sample was well suspended with 50 mM phosphate buffer (pH 7.0) and spread on one-half-strength modified-R2A agar (0.25 g tryptone, 0.25 g peptone, 0.25 g yeast extract, 0.125 g malt extract, 0.125 g beef extract, 0.25 g casamino acid, 0.25 g soytone, 0.5 g dextrose, 0.3 g soluble starch, 0.2 g xylan, 0.3 g sodium pyruvate, 0.3 g K_2HPO_4, 0.05 g $MgSO_4$, 0.05 g $CaCl_2$, agar 15 g, per 1 L) plates. The strain Gsoil 142^T was one of the isolates that appeared on the modified one-half-strength R2A agar plates under an aerobic condition. After isolation, it was routinely cultured on R2A agar (Difco, USA) at 30°C and maintained as a glycerol suspension (20%, w/v) at -70°C.

Gram-reaction was performed by the non-staining method described by Buck (1982). Cell morphology and motility were observed at ×1,000 magnification, with a light microscope (BX50, Olympus, Japan) and with transmission electron microscopy, using cells grown for 3 days at 30°C on R2A agar. For the latter, the cells were negatively stained with 1% (w/v) phosphotungstic acid and, after air-drying, grids were examined with a transmission electron microscope (model CM-20: Philips, Netherland). Catalase activity was determined by bubble production in 3% (v/v) H_2O_2 and oxidase activity was determined using 1% (w/v) tetramethyl *p*-phenylenediamine. For single-carbon-source assimilation studies, a defined liquid basal salt medium containing (g/L): K_2HPO_4 1.8 g, KH_2PO_4 1.08 g, $NaNO_3$ 0.5 g, NH_4Cl 0.5 g, KCl 0.1 g, $MgSO_4$ 0.1 g, and $CaCl_2$ 0.05 g was used. To this medium, a vitamin solution (Widdel and Bak, 1992), trace elements solution SL-10 (Widdel *et al.*, 1983) and a selenite/tungstate solution (Tschech and Pfennig, 1984) were added and the pH of the medium was adjusted to 6.8. This liquid medium was aliquoted (0.25 ml) into 96-well trays and filter-sterilized carbon sources were added to each well [individually at 0.1% (w/v)]. Growth in the 96-well plates was examined visually, and they were incubated at 30°C for 7 days. Negative control wells did not contain an added carbon source. Positive control culture was grown in

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wells containing R2A. In addition, several biochemical tests were carried out using API 20E galleries according to the manufacturer's instructions (bioMérieux, France). Anaerobic growth was performed in serum bottles after adding thioglycolate (1 g/L) to R2A broth and substituting the upper air layer with nitrogen gas. Aerobic nitrate reduction was subsequently confirmed by inoculation into 12 ml R2A broth supplemented with 10 mM KNO_3 in three 25 ml serum bottles. The reduction of nitrate was monitored by an ion chromatograph (Model 790 personal IC; Metrohm, Switzerland) equipped with a conductivity detector and an anion exchange column (Metrosep Anion Supp 4; Metrohm). Tests for DNA degradation [DNase agar, Scharlau (Spain) with via DNAse activity by flooding plates with 1 M HCl], casein, chitin, starch (Atlas, 1993), lipid (Kouker and Jaeger, 1987), xylan and cellulose (Ten *et al*., 2004) were performed and the results evaluated after 10 days. The effect of pH (pH 5.0-10.0 at intervals of 0.5 pH units) on growth was determined on R2A broth media using three different buffers at a final concentration of 50 mM: acetate buffer, pH range 5.0-5.5; phosphate buffer, pH range 6.0-8.0; Tris buffer, pH range 8.5-10.0. They were all filter-sterilized and assessed after 5 days of incubation. Salt tolerance was tested on R2A medium supplemented with 1-10% (w/v at intervals of 1.0% units) NaCl after 5 days of incubation. Growth on nutrient agar, trypticase soy agar (TSA), and MacConkey agar was also valuated at 30°C.

An almost-complete length of 16S rRNA gene sequence for strain Gsoil 142^T was determined as described below. Genomic DNA was extracted using a commercial genomic DNA-Extraction kit (Solgent, South Korea). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer set, comprising 9F and 1512R and the purified PCR products were sequenced by Solgent Co. Ltd (Daejeon, Korea) (Ten *et al*., 2008). The full length of sequence of the 16S rRNA gene was compiled using SeqMan software (DNASTAR, USA). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database and Eztaxon server (Chun *et al*., 2007). The multiple alignments were performed by using the CLUSTAL X program (Thompson *et al*., 1997). Gaps were edited in the BioEdit program (Hall, 1999). The evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). The phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) and maximum-parsimony (Fitch, 1971) in the MEGA4 Program (Kumar *et al.*, 2008), with bootstrap values based on 1,000 replications (Felsenstein, 1985).

For the measurement of G+C content of the chromosomal DNA, genomic DNA of the novel strain was extracted and purified as described by Moore and Dowhan (1995), and was enzymatically degraded into nucleosides, and was determined as described by Mesbah *et al.* (1989) using a reverse-phase HPLC. 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 (Hiraishi *et al*., 1996). Cellular fatty acids profiles were determined for strain Gsoil 142^T grown on R2A agar at 28°C for 5 days. The cellular fatty acids were saponified, methylated, and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acids analyzed by a gas chromatograph (Hewlett Packard 6890) were identified by the Microbial Identification software package (Sasser, 1990). Polar lipids were extracted, examined by two-dimensional TLC and identified as described by Minnikin *et al*. (1977).

Cells of strain Gsoil 142^T were found to be Gram-negative, strictly aerobic, non-spore-forming, and rods that were motile by means of a single polar flagellum (Fig. 1). The colonies grown on R2A agar plates for 3 days were smooth, flat, white, and irregularly shaped with a diameter of 1-2 mm. On R2A agar, the strain Gsoil 142^T was able to grow at 15-42°C but not at 4 or 45°C; the optimal growth temperature was 30°C. Carbon assimilation tests using API strips were all negative; however, these tests showed clear results for a single carbon assimilation test based on a basal salt medium. The physiological characteristics of the strain Gsoil 142^T are summarized in the genus and species descriptions, and a comparison of its selective characteristics with those of its nearest phylogenetic neighbor is given in Table 1.

The 16S rRNA gene sequences of the strain Gsoil 142^T determined in this study was continuous stretches of 1,477 bp (base position 17-1508 with respect to the *Escherichia coli* numbering system) and deposited in GenBank database (accession number AB257720). Preliminary comparison using the 16S rRNA gene sequences in GenBank indicated that strain Gsoil 142^T belonged to the family *Sinobacteraceae* in the order *Xanthomonadales* of the *Gammaproteobacteria.* Sequence similarity calculation using the EzTaxon server (http://www.eztaxon.org/; Chun *et al*., 2007) indicated that the closest relatives of strain were *Hydrocarboniphaga effusa* ATCC BAA 332^T (94.4%), *Nevskia ramosa* DSM 11499^T (94.1%), and *Alkanibacter difficilis* MN154.3^T (92.0%). This relationship between strain Gsoil 142^T and other members of the *Gammaproteobacteria* was also evident in the phylogenetic tree (Fig. 2). Strain Gsoil 142^T and 3 strains discussed above formed a monophyletic clade with a high bootstrap value (97%).

The G+C content of genomic DNA of strain Gsoil 142^T

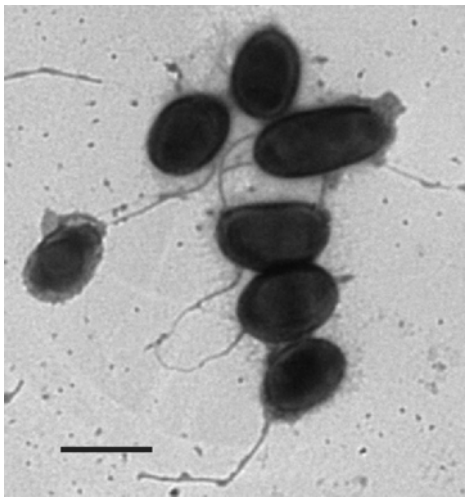

Fig. 1. Transmission electron micrograph of cells of strain Gsoil 142^T grown on R2A agar for 3 days. Bar=2 μm

Table 1. Differential characteristics of *Panacagrimonas perspica* gen. nov., sp. nov. and its nearest neighbors of the *Gammaproteobacteria*.
Strains: 1, Panacagrimonas perspica Gsoil 142^T; 2, Hydrocarboniphaga effusa ATCC BAA 332^T; 3, *Nevskia ramosa* DSM 11499^T.
All data were from this study unless indicated. All strains showed the positive activities for assimilation of D-fructose, propionate, 3-hydroxybutylate, valerate, fumaric acid, and D-sucrose. All strains showed the negative activities for assimilation of L-arabinose, D-lyxose, D-ribose, D-xylose, L-xylose, *N*-acetyl-glucosamine, benzoic acid, 3-hydroxy-benzoate, 4-hydroxy-benzoate, salicin, tartaric acid, itaconate, oxalic acid, D-lactose, D-maltose, D-melibiose, D-trehalose, D-raffinose, gluconate, dulcitol, inositol, D-sorbitol, xylitol, amygdalin, methanol, glycogen, dextran, arginine, asparagine, cysteine, glycine, lysine, methionine, serine, threonine, tryptophane, and tyrosine and hydrolysis of cellulose and skim milk. +, positive; -, negative

Characteristics	1	2	3
Oxidase	+	-	+
Hydrolysis of			
Xylan	-	+	-
Starch	-	+	-
Chitin	-	+	-
Assimilation of			
D-Galactose	+	-	-
D-Glucose	+	-	+
D-Mannose	+	-	-
D-Fucose	+	-	-
Ethanol	-	+	+
L-Rhamnose	+	+	-
L-Sorbose	+	-	-
D-Arabinose	+	-	-
Pyruvic acid	-	+	-
Fomic acid	+	+	-
Acetate	+	+	-
Caprate	-	+	+
Maleic acid	-	-	-
Phenyl-acetate	+	-	-
Citrate	-	+	-
Lactate	-	+	+
Malate	+	+	-
Malonate	+	-	+
Succinic acid	+	+	-
Glutaric acid	+	-	-
Adipate	+	-	+
Suberate	+	-	-
D-Cellobiose	+	-	-
D-Adonitol	+	-	-
D-Mannitol	-	+	-
Glycerol	+	-	+
Inulin	-	+	+
Alanine	-	-	+
Aspartic acid	+	-	+
Glutamic acid	+	+	-
Glutamine	+	-	-
Histidine	-	+	-
Iso-leucine	+	+	-
Leucine	-	+	-
Phenylalanine	-	+	-
Proline	+	-	-
Valine	+	+	-
DNA G+C (mol%)	69.9	60.0-61.0[a]	67.8[b]

[a] Data are taken from Palleroni *et al.* (2004).
[b] Data are taken from Stürmeyer *et al.* (1998).

Table 2. Cellular fatty acid profiles of strain Gsoil 142^T and related type strains of species.
Strains: 1, Panacagrimonas perspica Gsoil 142^T; 2, Hydrocarboniphaga effusa ATCC BAA 332^T; 3, *Nevskia ramosa* DSM 11499T.
All data were from this study. Cells were cultured on R2A agar for 5 days at 28˚C. Fatty acids representing less than 1.0% were omitted.

Fatty acid	1	2	3
$C_{12:0}$	11.2	-	7.3
$C_{12:0}$ 2-OH	4.5	-	-
$C_{12:0}$ 3-OH	3.5	2.4	2.9
$C_{13:0}$ 2-OH	-	-	1.4
$C_{14:0}$	2.3	2.3	6.5
$C_{14:0}$ 2-OH	-	1.0	2.5
$C_{16:0}$	17.4	14.8	5.0
$C_{17:0}$ anteiso	-	19.8	11.2
$C_{18:0}$ 3-OH	-	5.6	-
$C_{19:0}$ cyclo $\omega 8c$	2.4	-	-
Summed feature[a]	-		
2	1.9	1.9	1.8
3	20.6	13.2	16.9
8	36.3	37.9	44.6

[a] Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed features consist of: 2, $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; 3, $C_{16:1}$ $\omega 7c$ and/or iso-$C_{15:0}$ 2OH; 8, $C_{18:1}$ $\omega 7c$ and/or $\omega 6c$

was 69.9 mol%. Strain Gsoil 142^T had Q-8 as its predominant respiratory quinone. The cellular fatty acids of strain Gsoil 142^T and related type strains are listed in the Table 2. Cellular fatty acids of strain Gsoil 142^T comprising more than 10.0% were summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $\omega 6c$, 36.3%), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ $\omega 7c$, 20.6%), $C_{16:0}$ (17.4%), and $C_{12:0}$ (11.2%). According to the Table 2, qualitative and quantitative differences in fatty acid content could be observed between strain Gsoil 142^T and its phylogenetically closest relatives. In particular, Gsoil 142^T differed from other recognized 2 species by the presence of $C_{12:0}$ 2-OH, $C_{19:0}$ cyclo $\omega 8c$ and the absence of $C_{13:0}$ 2-OH, $C_{14:0}$ 2-OH, $C_{17:0}$ anteiso, and $C_{18:0}$ 3-OH. The polar lipids detected were phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, an unknown glycolipid, unknown aminophospholipids, unknown phospholipids, and an unknown lipid (Supplementary data Fig. 1). According to the Supplementary data Figs. 1-3, qualitative difference in polar lipids could be observed between strain Gsoil 142^T and its phylogenetically closest relatives.

Phylogenetically, the strain Gsoil 142^T forms a novel lineage of descent within the *Gammaproteobacteria* and clustered with *Hydrocarboniphaga effusa* ATCC BAA 332^T (94.4%), *Nevskia ramosa* DSM 11499^T (94.1%), and *Alkanibacter difficilis* MN154.3^T (92.0%). These low 16S rRNA gene sequence similarities support the allocation of the Gsoil 142^T strain into a novel genus. This is also supported by its phenotypic and chemotaxonomic characteristics (Tables 1 and 2). The Gsoil 142^T strain can be differentiated from *Hydrocarboniphaga effusa* ATCC BAA 332^T on the basis of its relatively high G+C content, its ability to grow at 42°C and to assimilate D-glucose, D-mannose and D-fucose, and by its inability to utilize lactate, ethanol, and inulin. This is presented in Table 1. Distinguishing

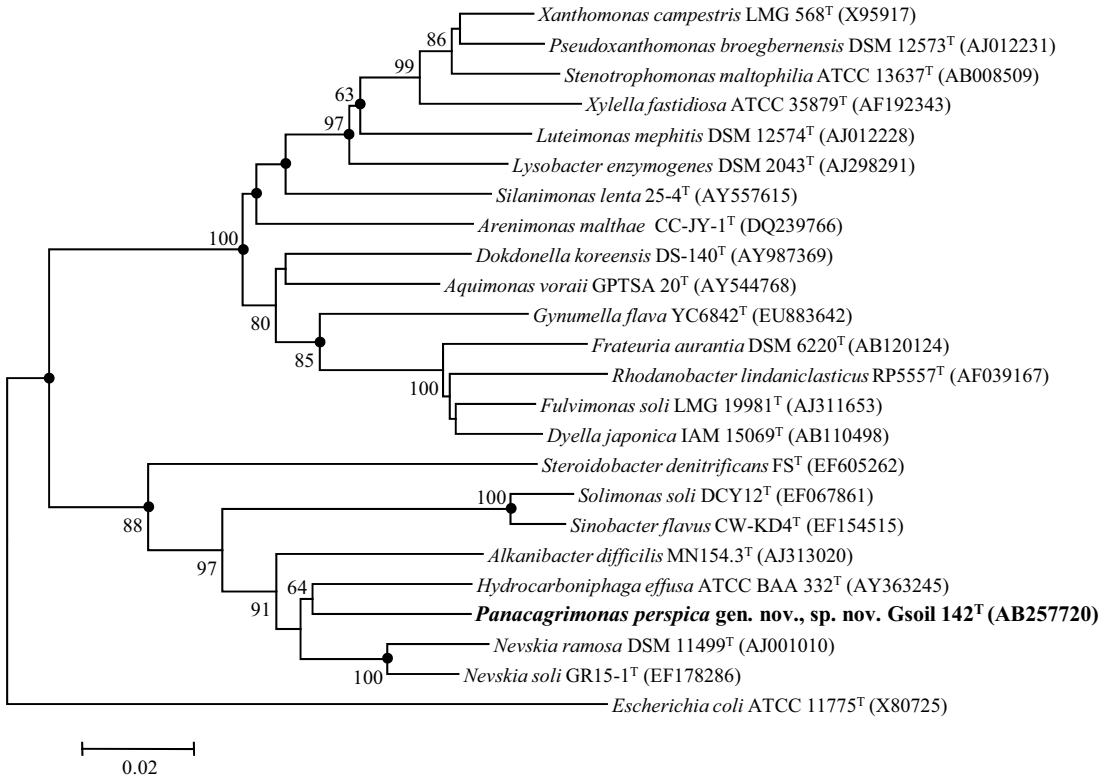

Fig. 2. Neighbour-joining tree showing the phylogenetic positions of *Panacagrimonas perspica* gen nov., sp. nov. and its nearest neighbours based on 16S rRNA gene sequence analysis. Dots indicate generic branches that were also recovered by using maximum parsimony algorithms. Bootstrap values (expressed as percentages of 1,000 replications) >60% are shown at the branch points. The tree was rooted by using *E. coli* as an outgroup. Bar=2 nucleotide substitutions per 100 nucleotide positions.

its phenotypic characteristics against *Nevskia ramosa* DSM 11499^T were its inability to assimilate caprate, ethanol and the other carbon substrates listed in Table 1, as well as its ability to produce oxidase and utilize D-galactose and D-arabinose as sole carbon sources.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparison, strain Gsoil 142^T is considered to represent a novel genus and species, for which the name *Panacagrimonas perspica* gen. nov., sp. nov. is proposed.

Description of *Panacagrimonas* gen. nov.

Panacagrimonas (Pa.na.ca.gri.mo'nas. N.L. fem. Panax -acis, scientific name of ginseng; ager agri, a field, monas a unit, monad; N.L. fem. gen. n. *Panacagrimonas*, monad of a ginseng field)

Cells are Gram-negative, rod-shaped (1.2-1.8 μm by 2.0-4.0 μm) and strictly aerobic. After 3 days incubation at 30°C on R2A, colonies are 1-2 mm in diameter, smooth, flat, white, and irregular shapes. Oxidase and catalase are positive. The major respiratory quinone is Q-8. The major fatty acids are summed feature 8 ($C_{18:1}$ *ω7c* and/or *ω6c*, 36.3%), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}$ *ω7c*, 20.6%), $C_{16:0}$ (17.4%), and $C_{12:0}$ (11.2 %). The polar lipids detected are phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, an unknown glycolipid, unknown aminophospholipids, unknown phosphorlipids, and an unknown lipid. NaCl is not needed for growth. The genus belongs to *Gammaproteobacteria.* The type species is *Panacagrimonas perspica*.

Description of *Panacagrimonas perspica* sp. nov.

Panacagrimonas perspica (pe.rsp.i´ca. L. fem. adj. perspica, bright and clear coloured)

In addition to the characteristics that define the genus, it has the following characteristics. Growth occurs between 15 and 42°C, the optimum temperature for growth is 30°C. The pH growth range is between pH 5.0 and 8.5, with an optimum between pH 6.5 and 7.0. Growth occurs in the absence of NaCl and in the presence of 7% (w/v) NaCl, but not in the presence of 8% (w/v) NaCl. No growth occurs on nutrient agar, TSA and MacConkey agar. Oxidase- and catalase- are positive. Does not reduce nitrate to nitrite and nitrite to nitrogen gas in aerobic conditions. Negative for hydrolyses of DNA, casein, chitin, starch, cellulose, xylan, and lipids. In API 20E tests, the Voges-Proskauer test, gelatinase, and tryptophane deaminase are positive but negative for ONPG test, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, citrate utilization, hydrogen sulphide, and indole production. Acid is not produced from D-glucose, D-melibiose, amygdalin, L-arabinose, D-mannitol, inositol, D-sorbitol, L-rhamnose, and D-sucrose.

The type strain, Gsoil 142^T (=KCTC 12982^T =LMG 23239^T) was isolated from soil of a ginseng field in Pocheon province, South Korea.

The NCBI GenBank accession number for the 16S rRNA gene sequence of strain Gsoil 142^T is AB257720.

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