Spirosoma panaciterrae sp. nov., isolated from soilLeonid N. Ten,¹ Jin-Li Xu,^{2,3} Feng-Xie Jin,³ Wan-Taek Im,² Hee-Mock Oh⁴ and Sung-Taik Lee²

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A Gram-negative, yellowish bacterial strain, designated Gsoil 1519^T, was isolated from soil of a ginseng field in Pocheon province (South Korea) and characterized using a polyphasic approach to determine its taxonomic position. Comparative 16S rRNA gene sequence analysis showed that strain Gsoil 1519^T belongs to the family 'Flexibacteraceae' and is related to *Spirosoma rigui* KCTC 12531^T (91.8 % similarity) and *Spirosoma linguale* LMG 10896^T (91.5 % similarity). Phylogenetic distances from any other recognized species within the family 'Flexibacteraceae' were greater than 14.7 %. The G + C content of the genomic DNA of strain Gsoil 1519^T was 50.1 %. The detection of MK-7 as the predominant menaquinone and a fatty acid profile with C_{16:1}ω5c, summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), iso-C_{15:0} and C_{16:0} as the major acids supported the affiliation of strain Gsoil 1519^T to the genus *Spirosoma*. On the basis of its phenotypic properties and phylogenetic distinctiveness, strain Gsoil 1519^T should be classified in the genus *Spirosoma* as a representative of a novel species, for which the name *Spirosoma panaciterrae* sp. nov. is proposed. The type strain is Gsoil 1519^T (=KCTC 22263^T=DSM 21099^T).

The genus *Spirosoma*, a member of the family 'Flexibacteraceae', phylum 'Bacteroidetes' (formerly *Flexibacter*–*Bacteroides*–*Flavobacterium*), was first proposed by Larkin & Borrall (1984). Currently, the genus contains two species, *Spirosoma linguale* (Larkin & Borrall, 1984) and *Spirosoma rigui* (Baik *et al.*, 2007). Strains of the genus have been isolated from soil and freshwater. During the course of an investigation of the culturable aerobic and facultatively anaerobic bacterial community in soil from a ginseng field in Pocheon province (South Korea), a Gram-negative, rod-shaped bacterial strain (Gsoil 1519^T) was isolated. On the basis of 16S rRNA gene sequence analysis, this isolate was considered to be a *Spirosoma*-like strain. To determine its exact taxonomic position, Gsoil 1519^T was subjected to a detailed investigation using a polyphasic taxonomic approach, including genotypic, chemotaxonomic and classical phenotypic analyses. These results indicate that Gsoil 1519^T should be placed in the genus *Spirosoma* as a representative of a novel species.

Strain Gsoil 1519^T was isolated from a soil sample from a ginseng field by using modified R2A medium as described previously (Ten *et al.*, 2006). Single colonies on the plates were purified by transferring them onto fresh plates of modified R2A agar followed by further incubation. Strain Gsoil 1519^T was purified from a colony growing on a modified R2A agar plate under aerobic conditions. It was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at –70 °C.

For phylogenetic analysis of strain Gsoil 1519^T, DNA was extracted using a genomic DNA extraction kit (Solgent), the 16S rRNA gene was amplified by PCR and sequencing of the purified PCR products was carried out according to Kim *et al.* (2005). Nearly complete 16S rRNA gene sequences were compiled using the SEQMAN software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed by 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). The phylogenetic tree was constructed by using neighbour-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 1519^T is EU370956.

joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA4 program (Tamura *et al.*, 2007) with bootstrap values based on 1000 replications (Felsenstein, 1985).

A nearly complete 16S rRNA gene sequence of strain Gsoil 1519^T was obtained (1442 bp). Preliminary sequence comparison with 16S rRNA gene sequences in GenBank indicated that strain Gsoil 1519^T belongs to the family 'Flexibacteraceae' within the phylum 'Bacteroidetes'. On the basis of 16S rRNA gene sequence similarity values, the closest cultured relatives were *S. rigui* KCTC 12531^T (91.8%) and *S. linguale* LMG 10896^T (91.5%). Phylogenetic distances from any other recognized species of the family 'Flexibacteraceae' were greater than 14.7% (i.e. sequence similarities were less than 85.3%). This relationship between strain Gsoil 1519^T and other members of the family 'Flexibacteraceae' was also evident in the phylogenetic tree (Fig. 1). Strain Gsoil 1519^T, *S. rigui* KCTC 12531^T and *S. linguale* LMG 10896^T formed a coherent cluster with high bootstrap values (100%) supported by the two separate tree-calculating methods employed in this study. The generally accepted criteria for delineating bacterial species state that strains with 16S rRNA gene sequence dissimilarity above 3% or strains with a DNA–DNA relatedness value of less than 70% (as measured by hybridization) are considered to belong to separate species (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Taking into account this definition, our data indicate that strain Gsoil 1519^T represents a novel species within the genus *Spirosoma*.

The Gram reaction of strain Gsoil 1519^T was determined by using the non-staining method described by Buck (1982). Cell morphology was observed under a Nikon light microscope at 1000× magnification with cells grown for 2 days at 30 °C on R2A agar. Catalase and oxidase tests were performed as outlined by Cappuccino & Sherman

(2002). Enzyme activities and other physiological characteristics were determined with API ZYM, API ID 32GN, API 20NE, API 50CH and API 20E galleries according to the manufacturer's instructions (bioMérieux). Tests for degradation of DNA [DNase agar (Scharlau), with DNase activity determined by flooding plates with 1 M HCl], casein, chitin, starch (Atlas, 1993), lipid (Kouker & Jaeger, 1987), xylan and cellulose (Ten *et al.*, 2004) were performed and evaluated after 5 days. Growth at various temperatures (4, 10, 15, 18, 25, 30, 37, 42 and 45 °C) and pH (pH 4.5–10.0 in increments of 0.5 pH units) was assessed on R2A agar and in R2A broth, respectively. Salt tolerance was tested on R2A medium supplemented with 1–10% (w/v) NaCl after 5 days of incubation. Growth on nutrient agar and trypticase soy agar (TSA) was also evaluated at 30 °C. For pigment analysis, cells were grown on R2A agar and spectral characteristics were performed by extraction with acetone according to Denner *et al.* (2001) using a Beckman DU 650 UV/visible spectrophotometer.

Cells of strain Gsoil 1519^T were Gram-negative, non-spore-forming, rod-shaped and non-motile, but had gliding activity. Colonies grown on R2A agar plates for 2 days at 30 °C were 1.0–1.5 mm in diameter, circular, convex, opaque, smooth and yellowish. In common with other recognized species of the genus *Spirosoma* (Baik *et al.*, 2007), strain Gsoil 1519^T was positive for catalase and oxidase activities, but negative for production of H₂S, indole and acetoin, reduction of nitrate to nitrite, and utilization of L-fucose, sorbose, dulcitol, glycerol, inositol, D-mannitol, D-sorbitol and citrate. Phenotypic and chemotaxonomic characteristics that differentiate strain Gsoil 1519^T from other members of the genus *Spirosoma* are listed in Table 1. In contrast to other *Spirosoma* species, strain Gsoil 1519^T grew at 42 °C, but not at 5 °C, and it was positive for gelatin hydrolysis and assimilation of L-arabinose and N-acetylglucosamine. The isolate produced

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain Gsoil 1519^T among neighbouring species selected from the phylum 'Bacteroidetes'. Bootstrap values (expressed as percentages of 1000 replications) greater than 60% are shown at branch points. Circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. *Bacillus subtilis* DSM 10^T (AJ276351) was used as an outgroup (not shown). Bar, 0.05 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of strain Gsoil 1519^T and recognized species of the genus *Spirosoma*

Strains: 1, Gsoil 1519^T; 2, *S. rigui* KCTC 12531^T; 3, *S. linguale* LMG 10896^T. Data are from Baik *et al.* (2007), Vancanneyt *et al.* (2006) and this study. Results for assimilation of sole carbon sources are based on API 20NE, API ID 32GN and API 50CH tests. All strains are positive for gliding motility and catalase. All strains are negative for Gram staining, H₂S, indole and acetoin production, reduction of nitrate to nitrite, and utilization of L-fucose, L-sorbose, dulcitol, glycerol, inositol, mannitol, sorbitol and citrate.

Characteristic	1	2	3
Growth at:			
42 °C	+	–	–
5 °C	–	+	+
NaCl range for growth (% w/v)	0–1	0–1	0–1.25
Oxidase	+	–	+
Hydrolysis of gelatin	+	–	–
β-Galactosidase (API ZYM)	–	+	+
Alkaline phosphatase (API ZYM)	+	–	+
Assimilation of:			
N-Acetylglucosamine	+	–	–
L-Arabinose	+	–	–
Cellobiose	+	–	+
Fructose	–	–	+
D-Galactose	–	–	+
D-Glucose	+	+	–
Maltose	+	–	+
Mannose	+	–	+
Melibiose	+	–	+
Raffinose	–	–	+
Rhamnose	–	–	+
Sucrose	+	–	+
D-Xylose	–	–	+
DNA G+C content (mol%)	50.1	53.3	51–53

yellow pigments with wavelength absorbance spectral peaks at 460 and 482 nm and a major peak at 460 nm, whereas the positions of the major peak and shoulder of its closest relative, *S. rigui* KCTC 12531^T, are at 451 and 478 nm, respectively (Baik *et al.*, 2007).

For the measurement of chromosomal DNA G+C content, the genomic DNA of the strain was extracted and purified as described by Moore & Dowhan (1995) and degraded enzymically into nucleosides; the DNA G+C content was determined as described by Mesbah *et al.* (1989) using reverse-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 quinone in n-hexane was purified using Sep-Pak Vac silica cartridges (Waters) and then analysed by HPLC, as described previously (Hiraishi *et al.*, 1996). Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI). The fatty acid methyl esters were then analysed by GC (model 6890;

Hewlett Packard) using the Microbial Identification software package (Sasser, 1990); ranges were obtained by duplicate experiments. Polar lipids were extracted and examined by two-dimensional TLC (Minnikin *et al.*, 1984).

Cellular fatty acid profiles of strain Gsoil 1519^T, *S. rigui* KCTC 12531^T and *S. linguale* LMG 10896^T are shown in Table 2. These three species contained C_{16:1}ω5c, summed feature 4 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH), iso-C_{15:0} and C_{16:0} as the predominant fatty acids. Major quantitative differences between strain Gsoil 1519^T and its closest neighbours were found in the amounts of the above-mentioned fatty acids. Apart from the latter differences, strain Gsoil 1519^T could be distinguished from other *Spirosoma* species by the presence of small amounts of C_{15:0} and by the absence of C_{16:0} 3-OH and iso-C_{15:0} 3-OH. Gsoil 1519^T contained MK-7 as the predominant menaquinone. The polar lipids detected were phosphatidylethanolamine (major lipid), diphosphatidylglycerol and two unknown lipids. These data are in good agreement with those of other members of the genus *Spirosoma* (Larkin & Borrall, 1984; Baik *et al.*, 2007). The DNA G+C content of strain Gsoil 1519^T was 50.1 mol%, which is slightly lower than values reported for *Spirosoma* species

Table 2. Cellular fatty acid content (%) of *Spirosoma panaciterrae* Gsoil 1519^T and related species of the genus *Spirosoma*

Strains: 1, *S. panaciterrae* sp. nov. Gsoil 1519^T (data from this study); 2, *S. rigui* KCTC 12531^T (Baik *et al.*, 2007); 3, *S. linguale* LMG 10896^T (Baik *et al.*, 2007). –, Not detected; tr, trace amount (<1 %).

Fatty acid	1	2	3
Straight-chain saturated:			
C _{14:0}	1.6	1.3	–
C _{15:0}	2.7	–	–
C _{16:0}	5.2	8.8	7.2
C _{18:0}	–	1.2	–
Branched saturated:			
iso-C _{15:0}	15.1	9.5	7.6
iso-C _{13:0}	–	–	3.8
anteiso-C _{15:0}	1.9	1.3	tr
Hydroxy fatty acids:			
C _{16:0} 3-OH	–	2.8	4.7
iso-C _{15:0} 3-OH	–	2.6	4.9
iso-C _{17:0} 3-OH	3.2	3.5	6.5
Mono-unsaturated:			
C _{16:1} ω5c	33.4	18.5	17.5
C _{18:1} ω7c	–	1.5	–
Summed feature 4*	35.5	45.6	47.9
Unknown (ECL 13.566)†	1.4	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 4 contains C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified.

(51.0–53.3 mol%). However, the value still lies within the range expected for members of the same genus and the G+C content range of the genus *Spirosoma* should be extended to take into account this result.

All of the characteristics determined for strain Gsoil 1519^T are in accordance with those of the genus *Spirosoma*. However, the phylogenetic distinctiveness of strain Gsoil 1519^T confirmed that this isolate represents a species that is distinct from recognized *Spirosoma* species. There are some phenotypic differences between strain Gsoil 1519^T and phylogenetically related *Spirosoma* species (Table 1). Therefore, on the basis of the data presented, strain Gsoil 1519^T should be classified within the genus *Spirosoma* as the type strain of a novel species, for which the name *Spirosoma panaciterrae* sp. nov. is proposed.

Description of *Spirosoma panaciterrae* sp. nov.

Spirosoma panaciterrae (pa.na.ci.ter'rae. N.L. n. *Panax* -*acis* scientific name for ginseng; L. n. *terra* soil; N.L. gen. n. *panaciterrae* of soil from a ginseng field, the source of the type strain).

Cells are Gram-negative, rod-shaped and do not form endospores. Non-diffusible yellow pigments are produced. Grows at 15–42 °C and pH 5.0–9.0; optimum growth occurs at 30 °C and pH 6.5–7.0. Does not require NaCl for growth, but can tolerate 1% (w/v) NaCl. Nitrate is not reduced to nitrite. Growth occurs on nutrient agar and TSA. Able to hydrolyse gelatin and starch (weakly), but not chitin, xylan, carboxymethylcellulose, casein or DNA. Produces *N*-acetyl- β -glucosaminidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, α -glucosidase, β -glucosidase, α -galactosidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase. Does not produce lipase (C14), valine arylamidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, urease, cystine arylamidase, trypsin, α -chymotrypsin, β -galactosidase, β -glucuronidase, α -mannosidase or α -fucosidase. The following substrates are utilized for growth: L-arabinose, D-glucose, D-mannose, cellobiose, maltose, melibiose, sucrose, *N*-acetylglucosamine and salicin. The following substrates are not utilized for growth: D-fructose, D-galactose, D-xylose, L-rhamnose, D-ribose, L-fucose, L-sorbose, raffinose, D-sorbitol, inositol, D-mannitol, dulcitol, glycerol, glycogen, acetate, citrate, gluconate, 3- or 4-hydroxybenzoate, malonate, itaconate, 2- or 5-ketogluconate, DL-3-hydroxybutyrate, valerate, malate, propionate, caprate, phenylacetate, adipate, lactate, suberate, L-alanine, L-histidine, L-proline or L-serine. The Voges–Proskauer reaction and tests for the production of H₂S and indole are negative. Acid is produced from D-mannose (weakly), D-sorbitol, aesculin ferric citrate, salicin (weakly), cellobiose, maltose and trehalose. Acid is not produced from glycerol, erythritol, D- or L-arabinose, D-ribose, D- or L-xylose, D-adonitol, methyl β -D-xylose, D-glucose, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, methyl α -D-mannopyranoside, methyl

α -D-glucopyranoside, amygdalin, arbutin, D-lactose, melibiose, sucrose, inulin, melezitose, starch, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D- and L-fucose, D- or L-arabitol, gluconate, or 2- or 5-ketogluconate. The major fatty acids are C_{16:1} ω 5c, summed feature 4 (C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH) and iso-C_{15:0}. The predominant menaquinone is MK-7. Phosphatidylethanolamine is the major polar lipid.

The type strain, Gsoil 1519^T (=KCTC 22263^T=DSM 21099^T), was isolated from soil of a ginseng field in Pocheon province (South Korea). The DNA G+C content of the type strain is 50.1 mol%.

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