Sphingomonas soli sp. nov., a β -glucosidase-producing bacterium in the family *Sphingomonadaceae* in the α -4 subgroup of the *Proteobacteria*

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Strain T5-04^T, a Gram-negative, non-spore-forming, rod-shaped bacterium, was isolated from soil of a ginseng field in South Korea and characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain T5-04^T belongs to the α -4 subgroup of the *Proteobacteria*, and the highest degrees of sequence similarity determined were to *Sphingomonas asaccharolytica* IFO 10564^T (97.5%), *Sphingomonas koreensis* JSS26^T (97.1%), *Sphingomonas mali* IFO 15500^T (96.7%) and *Sphingomonas pruni* IFO 15498^T (96.6%). Chemotaxonomic data revealed that strain T5-04^T possesses ubiquinone Q-10 predominantly, C_{18:1} as the predominant fatty acid and sphingoglycolipids, all of which corroborate its assignment to the genus *Sphingomonas*. The results of DNA–DNA hybridization and physiological and biochemical tests clearly demonstrated that strain T5-04^T represents a distinct species. Based on polyphasic evidence, T5-04^T (=KCTC 12210^T=NBRC 100801^T=IAM 15213^T) should be classified as the type strain of a novel *Sphingomonas* species, for which the name *Sphingomonas soli* sp. nov. is proposed.

The genus *Sphingomonas* was created by Yabuuchi *et al.* (1990) in order to accommodate Gram-negative, strictly aerobic, chemoheterotrophic, yellow-pigmented and rod-shaped bacteria that harbour sphingoglycolipids as cell-envelope components. The genus contained a multitude of species consisting of strains of clinical origin, as well as from a variety of other environments. The relatively diverse species of the genus *Sphingomonas* have been classified into at least four clusters within the α -4 subgroup of the *Proteobacteria*. The genus *Sphingomonas* has also been divided into four genera: *Sphingomonas sensu stricto*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* (Takeuchi *et al.*, 2001).

Yabuuchi *et al.* (2002) suggested that the genus *Sphingomonas* should remain undivided and that the species of *Novosphingobium*, *Sphingobium* and *Sphingopyxis* constitute later homotypic synonyms of species of the genus *Sphingomonas*. Subsequently, Busse *et al.* (2003) suggested

that sym-homospermidine, which is a characteristic marker within the family *Sphingomonadaceae*, had not been considered in the taxonomic considerations of Yabuuchi *et al.* (1990, 2002) and strongly advocated the proposal of Takeuchi *et al.* (2001). Nowadays, a great many articles have adopted the nomenclature of Takeuchi *et al.* (2001) in their reports of novel species in the genera *Novosphingobium* (Fujii *et al.*, 2003; Sohn *et al.*, 2004; Tirola *et al.*, 2005), *Sphingobium* (Ushiba *et al.*, 2003), *Sphingomonas* (Busse *et al.*, 2003; Li *et al.*, 2004; Rivas *et al.*, 2004) and *Sphingopyxis* (Kämpfer *et al.*, 2002; Godoy *et al.*, 2003; Yoon & Oh, 2005; Yoon *et al.*, 2005). At the time of writing, the genus *Sphingomonas sensu stricto* contains 28 species with validly published names.

In this study, a strain was isolated from soil of a ginseng field in Daejeon city in South Korea and characterized by polyphasic approaches. The results obtained in this study demonstrate that strain T5-04^T can be considered to be a member of the genus *Sphingomonas*, using the nomenclature provided by Takeuchi *et al.* (2001). However, it can also

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain T5-04^T is AB166883.

be clearly distinguished from *Sphingomonas* species with validly published names; we therefore propose that strain T5-04^T should be considered the type strain of a novel *Sphingomonas* species.

Strain T5-04^T was isolated from soil of a ginseng field via direct plating onto R2A agar (Difco). Single colonies on these plates were purified by transferring them onto new plates and subjecting them to an additional incubation for 3 days at 30 °C. One isolate, T5-04^T, was routinely cultured on R2A agar at 30 °C and maintained as a glycerol suspension (20 %, w/v) at -70 °C.

Extraction of genomic DNA was done using a commercial genomic DNA extraction kit (Core Biosystem). The 16S rRNA gene was amplified from the chromosomal DNA using the universal bacterial primer set 9F and 1512R and purified PCR products were sequenced by GenoTech Co. Ltd (Daejeon, South Korea) (Kim *et al.*, 2005). Full 16S rRNA gene sequences were compiled using SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from the GenBank database. Multiple alignments were performed with 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 a neighbour-joining method (Saitou & Nei, 1987) and the maximum-parsimony method (Fitch, 1972) using the MEGA3 program (Kumar *et al.*, 2004) with bootstrap values based on 1000 replications (Felsenstein, 1985).

The 16S rRNA gene sequence of strain T5-04^T was found to be a continuous stretch of 1422 nucleotides. Strain T5-04^T was determined to belong to the α -4 subgroup of the *Proteobacteria*, and the highest degrees of sequence similarity were found to be with *Sphingomonas asaccharolytica* IFO 10564^T (97.5 %), *Sphingomonas koreensis* JSS26^T (97.1 %), *Sphingomonas mali* IFO 15500^T (96.7 %) and *Sphingomonas pruni* IFO 15498^T (96.6 %). In the phylogenetic tree (Fig. 1), strain T5-04^T clearly belonged to the *Sphingomonas* lineage, as confirmed by the high bootstrap value.

Cell morphology and motility were observed with a Nikon light microscope (1000 $\times$ magnification), with the cells allowed to grow on R2A agar for 3 days at 30 °C. Gram reactions were conducted according to the non-staining method described by Buck (1982). Catalase activity was determined by bubble production in 3 % (v/v) H₂O₂ and oxidase activity was determined using 1 % (w/v) tetramethyl *p*-phenylenediamine. Acid production from carbohydrates was assessed by the procedures outlined by Cappuccino & Sherman (2002). Growth at a variety of temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar and growth at a variety of pH values was assessed in R2A broth. Substrate utilization as sole carbon source and some physiological characteristics were determined with API 32GN and API 20NE galleries according to the instructions of the manufacturer (bioMérieux).

When strain T5-04^T was cultured on R2A agar (Difco) at 30 °C, it produced yellowish coloured, circular and non-glossy colonies. Cells were aerobic, Gram-negative, non-motile rods. The strain was able to grow at 15–37 °C, but did not grow at 4 or 42 °C. Results regarding the physiological characteristics of strain T5-04^T are summarized in the species description and a comparison of selective characteristics with related type strains is shown in Table 1.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified via TLC and subsequently analysed by HPLC as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). Cellular fatty acids were analysed in organisms grown on trypticase soy agar (TSA; Difco) for 2 days. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The fatty acids analysed by GC (Hewlett Packard 6890) were identified by the Microbial Identification software package. Total cellular lipids were extracted three times from 50 mg dried cells with 5 ml chloroform/methanol (2:1, v/v) and a portion of the extracted lipids was subjected to mild alkaline hydrolysis with 0.5 M KOH in chloroform/methanol (2:1, v/v) for 1 h at 40 °C as described by Yabuuchi *et al.* (1990). Both total extracted lipids and their alkaline hydrolysates were analysed

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationships between strain T5-04^T and related species (neighbour-joining method). Bar, 0.01 substitutions per nucleotide position. Dots indicate branches that were also recovered by using the maximum-parsimony algorithm. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points.

Table 1. Differential phenotypic characteristics between strain T5-04^T and related taxa

Taxa: 1, *Sphingomonas soli* sp. nov. T5-04^T; 2, *Sphingomonas asaccharolytica* IFO 10564^T (data from Takeuchi *et al.*, 1995); 3, *Sphingomonas koreensis* JSS26^T (Lee *et al.*, 2001); 4, *Sphingomonas mali* IFO 15500^T (Takeuchi *et al.*, 1995); 5, *Sphingomonas pruni* IFO 15498^T (Takeuchi *et al.*, 1995); 6, *Sphingomonas paucimobilis* (Yabuuchi *et al.*, 1990). +, Positive; –, negative; W, weak reaction; ND, not determined. PNPG, *p*-Nitrophenyl α -D-glucopyranoside. All strains are Gram-negative, yellow, rod-shaped bacteria and are positive for assimilation of D-glucose and maltose. All strains are negative for nitrate reduction, indole production, arginine dihydrolase and urease activity and assimilation of 2-ketogluconate, 3-hydroxybenzoate, caprate, citrate, itaconate, malonate, phenylacetate, propionate, D-ribose, *myo*-inositol, D-mannitol and D-sorbitol.

Characteristic	1	2	3	4	5	6
Motility	–	+	+	+	+	–
Enzyme activities:						
Oxidase	+	+	+	+	+	–
Protease (gelatin hydrolysis)	+	–	–	–	–	+
β -Galactosidase (PNPG hydrolysis)	–	+	+	+	+	+
β -Glucosidase (aesculin hydrolysis)	+	+	–	+	+	+
Carbon assimilation:						
3-Hydroxybutyrate	–	ND	ND	+	+	ND
4-Hydroxybenzoate	–	ND	ND	+	–	ND
5-Ketogluconate	–	ND	–	+	–	ND
Acetate	+	–	ND	+	W	–
Adipate	–	–	–	+	–	–
Gluconate	–	–	–	+	+	+
DL-Lactate	–	–	ND	–	–	ND
Malate	–	–	+	+	–	+
Suberate	–	ND	ND	+	+	ND
Valerate	–	ND	ND	–	+	ND
L-Arabinose	+	+	–	+	+	+
L-Fucose	–	ND	ND	+	+	ND
D-Mannose	–	+	–	+	+	+
D-Melibiose	–	+	+	+	–	+
L-Rhamnose	–	+	+	+	+	+
Sucrose	+	–	+	+	+	+
L-Alanine	–	ND	ND	+	+	–
L-Histidine	–	ND	ND	+	+	ND
<i>N</i> -Acetylglucosamine	–	+	+	+	+	+
Salicin	–	–	+	+	+	+
L-Proline	–	ND	ND	–	–	ND
L-Serine	–	ND	ND	–	–	ND
DNA G + C content (mol%)	63.9	64.8	66	65.4–65.9	65.4	63.7

by TLC (HPTLC plate, Silica gel 60, 10 × 10 cm; Merck) with a solvent system composed of chloroform, methanol and water (70:30:5, by vol.). To detect glycolipid spots, orcinol-sulfuric acid reagent (0.2 % orcinol in 2 M sulfuric acid) was sprayed and charred at 120 °C until the maximum purple colour developed.

The genomic DNA of strain T5-04^T was extracted and purified with the Qiagen Genomic-tip system 100/G and was then enzymically degraded into nucleosides, as described previously (Mesbah *et al.*, 1989). DNA–DNA hybridization was performed fluorometrically, according to the method developed by Ezaki *et al.* (1989), using photobiotin-labelled

DNA probes and microdilution wells. Hybridization was conducted in five replications for each sample. The highest and lowest values obtained for each sample were excluded and DNA relatedness values are expressed as the means of the remaining three values.

The cellular fatty acid profiles of strain T5-04^T and related *Sphingomonas* type strains are shown in Table 2. The major cellular fatty acids in strain T5-04^T included summed feature 7, which includes isomers of octadecenoic acid (C_{18:1}, 65.5 %), and hexadecanoic acid (C_{16:0}, 14.3 %). Minor amounts of the 2-hydroxy fatty acids C_{14:0} 2-OH (3.4 %) and C_{15:0} 2-OH (0.9 %) were also determined to be present.

Table 2. Cellular fatty acid profiles of strain T5-04^T and related taxa

Taxa: 1, *Sphingomonas soli* sp. nov. T5-04^T; 2, *Sphingomonas asaccharolytica* IFO 10564^T; 3, *Sphingomonas koreensis* JSS26^T; 4, *Sphingomonas mali* IFO 15500^T; 5, *Sphingomonas pruni* IFO 15498^T; 6, *Sphingomonas paucimobilis*. Data were obtained in this study with the exception of column 6 (taken from Li *et al.*, 2004). Values are percentages of total fatty acids. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids						
C _{14:0}			1.4	16.8	4.9	1.4
C _{15:0}	0.8	3.8	0.2		2.8	
C _{16:0}	14.3	7.5	10.9	16.9	19.3	8.7
C _{17:0}		8.9			4.3	
C _{18:0}	0.5	0.4	0.5		2.6	
Unsaturated fatty acids						
C _{16:1}			1.4			
C _{17:1}	9.6	27.5	1.7	4.9	12.6	3.0
C _{18:1ω5c}			1.8		2.8	3.2
Summed feature 7*	65.5	50.2	69.2	58.4	50.1	74.6
Hydroxy fatty acids						
C _{14:0} 2-OH	3.4	0.4	9.8			6.4
C _{15:0} 2-OH	0.9	1.0	0.3			
C _{16:0} 2-OH			0.8			
Summed feature 4*	3.9		2.1	2.5		2.7

*Summed features designate groups of two or three fatty acids which cannot be separated by GLC with the MIDI system. Summed feature 4 contains C_{16:1 ω 7c} and/or C_{15:1} iso 2-OH. Summed feature 7 contains C_{18:1 ω 7c}, C_{18:1 ω 9t} and/or C_{18:1 ω 12t}.

The presence of 2-OH fatty acids and absence of 3-OH fatty acids and the presence of octadecenoic acid isomers as major fatty acids are characteristic features of members of the genus *Sphingomonas*, as has been discussed previously (Godoy *et al.*, 2003). Significant differences in fatty acid profiles were found in *Sphingomonas asaccharolytica*, *Sphingomonas koreensis*, *Sphingomonas mali* and *Sphingomonas pruni*. Q-10 was the predominant ubiquinone of strain T5-04^T. The quinone system supported our assignment of strain T5-04^T to the *Alphaproteobacteria*, in which the majority of species (including *Sphingomonas* species) exhibit Q-10 as the predominant quinone (Collins & Jones, 1981). Strain T5-04^T was also determined to harbour sphingolipids.

The G + C content of the genomic DNA of strain T5-04^T was 63.9 mol%. Strain T5-04^T exhibited relatively low levels of DNA–DNA relatedness to the type strains *Sphingomonas asaccharolytica* IFO 10564^T (22 %), *Sphingomonas koreensis* JSS26^T (23 %), *Sphingomonas mali* IFO 15500^T (20 %) and *Sphingomonas pruni* IFO 15498^T (12 %). The DNA–DNA hybridization level to related type strains was therefore less

than 70 % (Stackebrandt & Goebel, 1994), which is the suggested threshold which delineates a genomic species. Our results therefore support the assignment of strain T5-04^T to a separate, previously unrecognized species.

On the basis of morphological, physiological and chemotaxonomic characteristics, together with data from 16S rRNA gene sequence comparisons, strain T5-04^T should be placed into a novel species, for which we propose the name *Sphingomonas soli* sp. nov.

Description of *Sphingomonas soli* sp. nov.

Sphingomonas soli (so'li. L. neut. gen. n. *soli* of soil, the source of the type strain).

Cells are Gram-negative, non-motile, non-spore-forming rods (approx. 1 μ m in length). They are oxidase-positive, exhibiting oxidative metabolism (obligately aerobic), and catalase-positive. Favourable growth occurs aerobically at 30 °C on R2A agar, nutrient agar and TSA; optimal growth occurs at pH 6.8–7.5. Forms yellowish, convex, circular colonies with whole edges on R2A agar within 3 days, approximately 1.5–3.0 mm in diameter. Q-10 is the predominant quinone. The major cellular fatty acids include octadecenoic acid (C_{18:1}) and hexadecanoic acid (C_{16:0}). Sphingoglycolipids are also present. The G + C content of the genomic DNA is 63.9 mol% (determined by HPLC). Carbon and nitrogen source utilization and enzyme activities are shown in Table 1.

The type strain, T5-04^T (= KCTC 12210^T = NBRC 100801^T = IAM 15213^T), was isolated from soil of a ginseng field in Daejeon, South Korea.

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