Solirubrobacter soli sp. nov., isolated from soil of a ginseng field

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A Gram-positive, non-spore-forming, rod-shaped and non-motile bacterium, strain Gsoil 355^T, was isolated from soil of a ginseng field in South Korea. In phylogenetic analyses based on 16S rRNA gene sequences, strain Gsoil 355^T showed the highest levels of sequence similarity with respect to *Solirubrobacter pauli* B33D1^T (97.4%), *Conexibacter woesei* DSM 14684^T (94.2%) and *Patulibacter minatonensis* KV-614^T (91.8%). The strain possesses menaquinone MK-7(H₄) and contains C_{16:0} and C_{18:0}ω9c as the predominant fatty acids. The DNA G+C content is 71.5 mol%. On the basis of genotypic and phenotypic characteristics, strain Gsoil 355^T represents a novel species of the genus *Solirubrobacter*, for which the name *Solirubrobacter soli* sp. nov. is proposed. The type strain is Gsoil 355^T (=KCTC 12628^T=LMG 23485^T).

Members of the phylum *Actinobacteria* are widespread in soils throughout the world. The genus *Solirubrobacter* and the species *Solirubrobacter pauli* were proposed by Singleton *et al.* (2003) for a member of the *Actinobacteria* isolated from a burrow of the earthworm *Lumbricus rubellus* in an agricultural soil in Georgia, USA (Furlong *et al.*, 2002). A novel strain of this genus, Gsoil 355^T, was found in soil from a ginseng field in Daejeon, South Korea.

Strain Gsoil 355^T was isolated by direct plating of the serially diluted soil sample onto R2A agar (Difco). Single colonies from these plates were transferred onto new plates and incubated for 5 days at 30 °C. The purified colonies were tentatively identified using partial 16S rRNA gene sequences. Cell morphology and motility were investigated (using a Nikon light microscope at × 1000 magnification) in a 5-day-old culture of Gsoil 355^T grown on R2A agar at 30 °C. The Gram reaction was tested using the non-staining method, as described by Buck (1982). Oxidase activity was evaluated via the oxidation of 1% *N,N,N',N'*-tetramethyl-*p*-phenylenediamine dihydrochloride. Catalase activity was determined by measuring bubble production after the

application of 3% (v/v) hydrogen peroxide solution to well-isolated colonies. Growth at a variety of temperatures (4, 15, 25, 30, 37 and 42 °C) and pH (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9 and 11) was assessed on R2A agar; the pH was adjusted by using HCl and NaOH. Growth at various salt concentrations was tested by adding 0–10% NaCl to R2A broth. The API 20NE, API ZYM and API ID 32GN microtest systems (bioMérieux) were used to analyse the physiological and biochemical characteristics, according to the recommendations of the manufacturer.

Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified by TLC and then analysed by HPLC as described previously (Collins & Jones, 1981; Shin *et al.*, 1996). For analysis of the fatty acid methyl esters, the strain was grown on tryptic soy agar (Difco) for 48 h at 30 °C and then two loops of the well-grown cells were harvested. Fatty acid methyl esters were prepared and identified using the Sherlock Microbial Identification System (MIDI) (Sasser, 1990).

The genomic DNA of strain Gsoil 355^T was extracted and purified with Genomic-tip system 100/G (Qiagen). It was then enzymically degraded into nucleosides to determine the DNA G+C content (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). DNA–DNA hybridization was performed according to the method developed by Ezaki *et al.* (1989). Hybridization was conducted using five replications for each sample. The highest and lowest values were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Gsoil 355^T is AB245334.

The cellular fatty acid profiles of strain Gsoil 355^T and related type strains are presented in a supplementary table available with the online version of this paper.

excluded and the DNA relatedness taken from the mean of the remaining three values.

For analysis of the 16S rRNA gene sequence, genomic DNA was extracted and purified with a genomic DNA isolation kit (Core Bio System). The 16S rRNA gene was amplified using the universal bacterial primers 9F and 1512R (Weisburg *et al.*, 1991) and the purified PCR products were sequenced by Genotec (Daejeon, Korea) and then edited (Kim *et al.*, 2005). The 16S rRNA gene sequences of related taxa were obtained from GenBank. Multiple alignments were performed with the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) in the MEGA2 program (Kumar *et al.*, 2001). A bootstrap analysis (based on 1000 replicates) was also performed (Felsenstein, 1985).

Strain Gsoil 355^T produced non-pigmented colonies on R2A agar at 30 °C. The strain comprised aerobic, Gram-positive, non-motile, rod-shaped bacteria. The optimal growth temperature was about 30 °C. No growth was observed within 7 days at 37 °C and only weak growth occurred after 14 days at 15 °C. The physiological characteristics are summarized in the species description.

The 16S rRNA gene sequence-based phylogenetic analysis showed (Fig. 1) that strain Gsoil 355^T is a member of the family *Solirubrobacteraceae* (Stackebrandt, 2004, 2005). The highest sequence similarities (97.4, 94.2 and 91.8 %) were found with respect to the type strains of *S. pauli* (Singleton *et al.*, 2003), *Conexibacter woesei* (Monciardini *et al.*, 2003) and *Patulibacter minatonensis* (Takahashi *et al.*, 2006), respectively.

The cellular fatty acid profiles of strain Gsoil 355^T and the related *Solirubrobacter*, *Conexibacter* and *Patulibacter* species are shown in Supplementary Table S1 (available in IJSEM Online). The major cellular fatty acids in strain Gsoil 355^T included C_{16:0} iso (26.7 %) and C_{18:1ω9c} (18.3 %). Minor amounts of fatty acids C_{14:0} (0.7 %), C_{14:0} iso (0.6 %), C_{15:0} (1.9 %), C_{16:0} (2.1 %), C_{17:0} (5.3 %), C_{18:0} (3.3 %), C_{18:0} iso (4.3 %), C_{19:0} (4.5 %), C_{20:0} (1.1 %), unsaturated fatty acids C_{16:1} iso H (3.0 %), C_{16:1ω9c}

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain Gsoil 355^T and related species in the genera *Solirubrobacter*, *Conexibacter* and *Patulibacter*. Bar, 0.02 substitutions per nucleotide position.

(0.9 %), C_{17:1ω6c} (5.5 %), C_{17:1ω8c} (4.3 %), C_{18:3ω6c} (7.3 %) and C_{20:4ω6,9,12,15c} (0.9 %), summed feature 4 (C_{16:1ω7c}/C_{15:0} iso 2-OH; 1.4 %) and summed feature 8 (unknown ECL 18.756/C_{19:1ω11c}; 7.9 %) were also detected. No significant differences were found between the fatty acid profiles of strain Gsoil 355^T and *S. pauli* B33D1^T (Singleton *et al.*, 2003).

Strain Gsoil 355^T contained a menaquinone with seven isoprene units [MK-7(H₄)] as the predominant isoprenoid quinone.

The DNA G+C content of strain Gsoil 355^T was 71.5 mol%. The strain exhibited 25.0 % (mean of 22, 29 and 27 %) DNA–DNA relatedness with respect to *S. pauli* ATCC BAA-492^T. The level of relatedness was less than 70 %, which is considered to be the threshold for delineating a genomic species (Wayne *et al.*, 1987). In addition, the strain could be readily distinguished from *S. pauli* at the phenotypic level (Table 1). The results of the polyphasic analysis clearly show that strain Gsoil 355^T represents a novel species within the genus *Solirubrobacter*, for which the name *Solirubrobacter soli* sp. nov. is proposed.

Description of *Solirubrobacter soli* sp. nov.

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

Cells are Gram-positive, aerobic, non-motile, non-pigmented rods. Growth occurs on R2A agar at 30 °C. Good growth is observed at 25 and 30 °C. Growth occurs at 0–1.5 % (w/v) NaCl but not at higher salt concentrations. Catalase-positive and weakly oxidase-positive. *N*-Acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase,

Table 1. Comparison of phenotypic characteristics of strain Gsoil 355^T and *S. pauli* ATCC BAA-492^T

Data for *S. pauli* ATCC BAA-492^T were taken from Singleton *et al.* (2003). Both strains were Gram-positive, non-motile rods and both were catalase-positive and unable to grow with more than 1.5 % NaCl. Both strains could utilize mannose and sucrose and neither could utilize citrate, 3-hydroxybenzoate, malate or mannitol. +, Positive; –, negative; w, weakly positive.

Characteristic	Gsoil 355 ^T	<i>S. pauli</i> ATCC BAA-492 ^T
Cell length (μm)	1.0–3.0	1.4
Colony colour	White	Pink
Oxidase activity	w	–
Growth at 1 % NaCl	+	–
Utilization of carbon sources		
Acetate	–	+
D-Sorbitol	–	+
L-Alanine	–	+
DNA G+C content (mol%)	71.5	71.8

cystine arylamidase, esterase (C4), esterase (C8), α -glucosidase, β -glucosidase, β -galactosidase, leucine arylamidase and valine arylamidase are produced. Arginine dihydrolase, α -chymotrypsin, α -fucosidase, α -galactosidase, β -glucuronidase, lipase (C14), β -mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and urease are not produced. Gelatin is hydrolysed. Adipate, gluconate, L-arabinose, L-fucose, D-glucose, D-maltose, D-melibiose, L-rhamnose, D-ribose, sucrose, *myo*-inositol, L-proline, *N*-acetyl-D-glucosamine, salicin and glycogen are utilized as sole carbon sources. 2-Ketogluconate, 3-hydroxybenzoate, 3-hydroxybutyrate, 4-hydroxybenzoate, 5-ketogluconate, acetate, caprate, citrate, itaconate, lactate, L-malate, malonate, phenyl acetate, propionate, suberate, *n*-valerate, D-mannitol, D-sorbitol, L-alanine and L-histidine are not utilized as sole carbon sources. No reduction of nitrate to nitrite or nitrogen gas occurs. The DNA G+C content is 71.5 mol% (HPLC). The predominant quinone is MK-7(H₄). The major cellular fatty acids are C_{16:0} iso (26.7 %) and C_{18:1 ω 9c} (18.3 %).

The type strain, Gsoil 355^T (=KCTC 12628^T=LMG 23485^T), was isolated from soil from a ginseng field in Daejeon, South Korea.

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