Sphingobacterium siyangense sp. nov., isolated from farm soil

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The taxonomic position of a novel Gram-negative strain, designated SY1^T, isolated from a farm-soil sample obtained from Jiangsu Province, PR China, was characterized by using a polyphasic approach. The cells were non-motile, non-spore-forming rods. The organism grew optimally at 30–37 °C and at pH 6.0–8.0. Based on 16S rRNA gene sequence analysis, strain SY1^T is a member of the genus *Sphingobacterium*; *Sphingobacterium multivorum* JCM 21156^T was the nearest relative (98.5 % sequence similarity). The predominant fatty acids of strain SY1^T were iso-$C_{15:0}$ (32.9 %), $C_{16:0}$ (10.9 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 24.1 %). The DNA G+C content was 38.5 mol%. The low level of DNA–DNA relatedness (2.2 %) to *S. multivorum* JCM 21156^T in combination with differential morphological and biochemical properties demonstrated that strain SY1^T (=KCTC 22131^T=CGMCC 1.6855^T) should be classified as representing a novel species of the genus *Sphingobacterium* for which the name *Sphingobacterium siyangense* sp. nov. is proposed.

The family *Sphingobacteriaceae* of the phylum *Bacteroidetes* (previously, the *Cytophaga–Flavobacterium–Bacteroides* group) currently comprises seven genera including *Sphingobacterium* and *Pedobacter* (Steyn *et al.*, 1998) and [*Flexibacter*] *canadensis* (a misclassified *Flexibacter* species). The type genus *Sphingobacterium* was proposed originally by Yabuuchi *et al.* (1983) to include Gram-negative rods that are positive for catalase and oxidase, negative for heparinase, gelatinase and indole production and having iso-$C_{15:0}$, iso-$C_{15:0}$ 2-OH, $C_{16:1}\omega7c$ and $C_{17:0}$ 3-OH as the main fatty acids. At the time of writing, the genus comprised eight recognized species, including *Sphingobacterium multivorum*, *S. spiritivorum* and *S. mizutaii* (Yabuuchi *et al.*, 1983) [*mizutae* was corrected to *mizutaii*; Holmes *et al.* (1988)], *S. antarcticum* (Shivaji *et al.*, 1992) [*antarcticus* was corrected to *antarcticum*; Euzéby (1998)], *S. thalpophilum* and *S. faecium* (Takeuchi & Yokota, 1992), *S. daejeonense* (Kim *et al.*, 2006) and *S. composti* (Yoo *et al.*, 2007).

A Gram-negative, aerobic, non-spore-forming bacterial strain (designated SY1^T) was isolated from a farm-soil sample and subjected to a polyphasic taxonomy approach. Based on the resulting data, strain SY1^T is considered to represent a novel species of the genus *Sphingobacterium*.

Strain SY1^T was isolated using the dilution-plating technique on Luria–Bertani (LB) agar (l^{-1}: 5 g yeast extract, 10 g tryptone, 10 g NaCl; pH 7.0) incubated at 30 °C and was cultivated routinely on LB agar or in LB broth at the same temperature under aerobic conditions. Stock cultures were maintained as a glycerol suspension (20 %, w/v) at −70 °C.

Amplification of the 16S rRNA gene sequence was performed as described by Cui *et al.* (2001). The sequence obtained was compared with available sequences retrieved from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/) to determine an approximate phylogenetic affiliation. Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 3.1 (Kumar *et al.*, 2001) after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Evolutionary distances (distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) and clustering were based on the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Bootstrap analysis (1000

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SY1^T is EU046272.

Transmission electron micrographs of cells of strain SY1^T are available as supplementary material with the online version of this paper.

replications) was used to evaluate the topology of the neighbour-joining tree (Felsenstein, 1985).

An almost complete 16S rRNA gene sequence (1489 bp) of strain SY1^T was obtained. The phylogenetic tree (Fig. 1) based on the neighbour-joining algorithm showed that strain SY1^T grouped among *Sphingobacterium* species and joined *S. multivorum* JCM 21156^T (98.5 % similarity) with a high bootstrap value of 100 %. Strain SY1^T shared <96.0 % 16S rRNA gene sequence similarity with other recognized *Sphingobacterium* species.

DNA–DNA hybridization was carried out to evaluate the genomic DNA–DNA relatedness between strain SY1^T and *S. multivorum* JCM 21156^T by using the optical renaturation rate method as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were computed with the program TRANSFER.BAS (Jahnke, 1992). Strain SY1^T exhibited low DNA–DNA relatedness with its closest relative *S. multivorum* JCM 21156^T (2.2 %). According to Stackebrandt & Goebel (1994) and Wayne *et al.* (1987), the levels obtained for 16 rRNA gene similarity (<97 %) and DNA–DNA relatedness (<70 %) support the genomic distinction of strain SY1^T from other *Sphingobacterium* species.

Morphological features were examined by using transmission electron microscopy (Hitachi-7650) as described by Nedashkovskaya *et al.* (2005). Gram staining and endospore-forming features were investigated using light microscopy (model BH 2; Olympus) according to the method described by Gerhardt *et al.* (1994). Anaerobic growth and growth at different temperatures and pH values were investigated according to Kim *et al.* (2006) and Xu *et al.* (2005), respectively, using LB as the basal medium. NaCl tolerance was tested using LB broth containing 0–5 % (w/v) NaCl. Growth was evaluated on cetrimide agar, Simmons' citrate agar, MacConkey agar and nutrient agar. All tests were incubated at 30 °C for 2 days. Oxidase activity was tested by determining the oxidation of 1 % (w/v) tetramethyl-*p*-phenylenediamine (Merck) and catalase activity was evaluated by determining the production of oxygen bubbles in a 5 % (v/v) aqueous hydrogen peroxide solution. Hydrolysis of casein, gelatin, aesculin, starch, carboxymethylcellulose, Tween 80 and Tween 20 was tested on LB agar after 2 days incubation as described by Lányí (1987) and Gerhardt *et al.* (1994). Degradation of DNA (using DNase agar from Scharlau, supplemented with 1 M HCl) and chitin was also investigated as described by Kim *et al.* (2006). Acid production from carbohydrates was determined using the medium and method described by

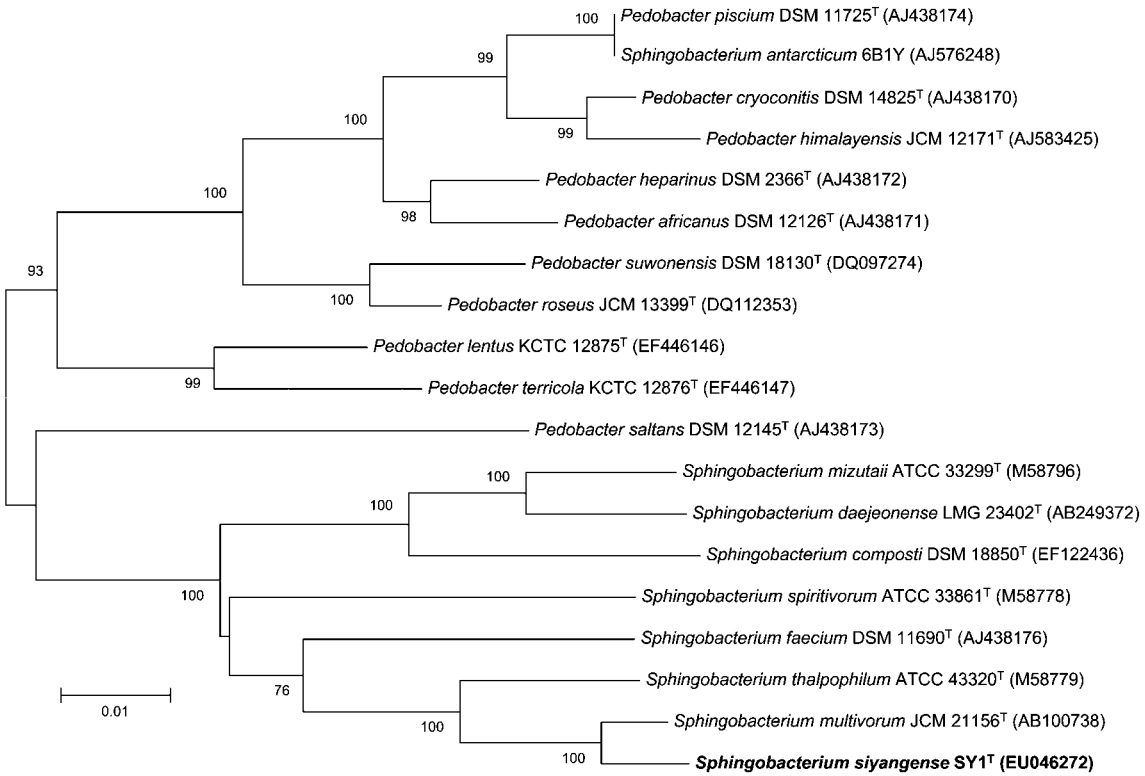

Fig. 1. Comparative analysis of the 16S rRNA gene sequences of strain SY1^T and representative strains of the genus *Sphingobacterium* using the neighbour-joining method. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branching points. The maximum-likelihood tree showed essentially the same topology (data not shown). Bar, 0.01 substitutions per nucleotide position.

Yamaguchi & Yokoe (2000). Single carbon-source utilization was determined as described by Zhou *et al.* (2007). Some other biochemical characteristics, as given in the species description below, were tested using API 20E kits (bioMérieux) according to the instructions of the manufacturer. Antibiotic sensitivity tests were performed using the diffusion method as described by Park *et al.* (2007) on LB agar at 30 °C with filter-paper discs (8 mm diameter; Sanofi Pasteur) containing the following antibiotics (μg): streptomycin (10), ampicillin (10), chloramphenicol (30), erythromycin (15), tetracycline (30), ofloxacin (5), rifampicin (5), gentamicin (10), ceftazidime (30), kanamycin (30), carbenicillin (100) and polymyxin B (30). After 2 days incubation, the diameters of the inhibition zones were measured. Phenotypic characteristics of strain SY1^T are given in the species description and in Table 1. Transmission electron micrographs of cells of strain SY1^T are shown in Supplementary Fig. S1 (available in IJSEM Online).

Genomic DNA was extracted and purified according to Yoon *et al.* (1996) and the DNA G+C content was determined using the thermal denaturation method (Mandel & Marmur, 1968). The G+C content of the DNA of strain SY1^T was 38.5 mol%, within the range of values reported for members of the genus *Sphingobacterium* (37.3–44.2 mol%; Yoo *et al.*, 2007).

For quantitative analysis of the cellular fatty acid content, cells were harvested after 2 days growth at 30 °C on trypticase soy agar and fatty acid methyl esters were prepared and identified by following the instructions of the Microbial Identification system (MIDI), as described by Sasser (1990). The cellular fatty acids mainly comprised iso-$C_{15:0}$ (32.9 %), $C_{16:0}$ (10.9 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$ 24.1 %), typical of members of the genus *Sphingobacterium* (Takeuchi & Yokota, 1992; Steyn *et al.*, 1998). The cellular fatty acids of strain SY1^T are listed in Table 2 and compared with those

Table 1. Differential characteristics of strain SY1^T and related *Sphingobacterium* species

Strains: 1, SY1^T (*S. siyangense* sp. nov.); 2, *S. multivorum* JCM 21156^T; 3, *S. thalpophilum* ATCC 43320^T; 4, *S. faecium* DSM 11690^T; 5, *S. mizutaii* ATCC 33299^T; 6, *S. daejeonense* LMG 23402^T; 7, *S. spiritivorum* ATCC 33861^T; 8, *S. composti* DSM 18850^T. Data were from Steyn *et al.* (1998), Kim *et al.* (2006), Yoo *et al.* (2007) and this study. +, Positive; –, negative; V, results vary between references; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Growth at:								
4 °C	+	–	–	+	–	–	–	–
42 °C	+	–	+	–	–	+	–	+
Hydrolysis of:								
DNA	+	+	–	+	+	–	+	–
Starch	+	+	+	+	+	–	+	–
Aesculin	–	+	+	+	+	–	+	+
Tween 80	–	V	–	V	+	ND	+	+
Urea	+	+	+	+	+	–	+	–
Tyrosine	–	V	–	–	+	–	–	–
Lysine	+	–	–	–	V	–	–	ND
Utilization of:								
D-Ribose	+	–	–	–	–	–	–	–
L-Sorbose	+	–	–	–	–	–	–	–
L-Rhamnose	+	–	+	+	–	–	+	–
L-Arabinose	+	+	+	+	V	–	–	+
D-Mannitol	+	–	–	–	–	–	+	–
Xylitol	+	–	–	–	–	–	–	–
Adonitol	+	–	+	–	–	+	–	–
Inulin	+	V	+	+	–	–	V	–
Gluconate	+	–	–	–	–	–	–	–
Glycerol	+	–	+	+	–	–	V	–
Acid production from:								
D-Glucose	–	+	+	+	+	+	+	–
Sucrose	–	+	+	+	+	–	+	–
Trehalose	+	–	–	–	–	–	–	+
L-Rhamnose	+	V	+	–	–	–	–	–
L-Arabinose	–	+	+	+	+	–	–	+
Nitrate reduction	+	–	+	–	–	ND	–	–
DNA G+C content (mol%)	38.5	39.9–40.5	44.0–44.2	37.3	39.3–40.0	38.7	39.8	42.3

Table 2. Fatty acid contents (%) of strain SY1^T and related *Sphingobacterium* species

Strains: 1, SY1^T (*S. siyangense* sp. nov.); 2, *S. multivorum* JCM 21156^T; 3, *S. thalpophilum* ATCC 43320^T; 4, *S. faecium* DSM 11690^T; 5, *S. mizutaii* ATCC 33299^T; 6, *S. daejeonense* LMG 23402^T; 7, *S. spiritivorum* ATCC 33861^T; 8, *S. composti* DSM 18850^T. Data from Steyn *et al.* (1998), Kim *et al.* (2006), Yoo *et al.* (2007) and this study. Fatty acids amounting to less than 1.0 % in all strains tested are not listed. tr, Trace (<1.0 %); –, not detected; ECL, equivalent chain-length.

Fatty acid	1	2	3	4	5	6	7	8
$C_{14:0}$	3.9	2.7	3.2	tr	tr	–	1.0	–
anteiso-$C_{15:0}$	2.7	–	–	tr	tr	2.6	tr	–
iso-$C_{15:0}$	32.9	22.2	17.7	24.6	30.0	45.6	30.1	29.5
iso-$C_{15:0}$ 3-OH	3.0	3.2	4.3	3.7	3.0	1.5	2.2	2.3
$C_{16:0}$	10.9	7.8	6.0	4.5	tr	3.4	3.5	2.2
$C_{16:0}$ 2-OH	tr	tr	3.2	–	–	–	–	–
$C_{16:0}$ 3-OH	6.4	5.3	6.3	2.1	tr	–	2.7	1.2
$C_{16:0}$ 10-methyl	–	–	–	1.4	–	–	–	–
$C_{16:1}\omega5c$	tr	–	–	1.5	tr	tr	tr	–
iso-$C_{17:0}$ 3-OH	5.9	7.1	10.0	10.0	22.1	16.6	12.5	19.7
iso-$C_{17:1}\omega9c$	1.1	tr	–	–	3.7	2.9	1.7	2.9
Summed feature 3*	24.1	49.0	47.8	48.1	35.1	23.8	42.7	37.5
ECL 13.566	tr	–	1.4	tr	1.3	1.0	tr	–

*Summed feature 3 contains iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

of the type strains of phylogenetically related *Sphingobacterium* species.

On the basis of phylogenetic evidence and phenotypic distinctiveness (Table 1), it is proposed that strain SY1^T should be classified in the genus *Sphingobacterium* as representing a novel species, for which the name *Sphingobacterium siyangense* sp. nov. is proposed.

Description of *Sphingobacterium siyangense* sp. nov.

Sphingobacterium siyangense (si.yang.en′se. N.L. neut. adj. *siyangense* pertaining to Siyang in Jiangsu Province, China, the city where the strain was isolated).

Cells are Gram-negative, non-motile, non-spore-forming, strictly aerobic rods, 0.8–1.7 μm long and 0.7–0.9 μm wide. After 2 days incubation on LB agar, colonies are 1.0–2.0 mm in diameter, slightly yellowish, convex, circular and smooth with entire margins. Grows at 4–42 °C (optimum, 30–37 °C), pH 3.0–10.0 (optimum, pH 6.0–8.0) and 0–4 % NaCl (optimum, 0–2 %). Growth occurs on nutrient agar and cetrimide agar, but not on Simmons' citrate agar or MacConkey agar. Positive for catalase and oxidase. In API 20E tests, positive for β-galactosidase, arginine dihydrolase, arginine decarboxylase, urease and nitrate reduction. Negative for ornithine decarboxylase, lysine decarboxylase, tryptophan deaminase, indole and H_2S production and Voges–Proskauer reaction. Starch, DNA, aesculin and Tween 20 are hydrolysed, but casein, gelatin, carboxymethylcellulose, Tween 80 or chitin are not. Acid is produced from trehalose and L-rhamnose, but not from D-glucose, cellobiose, D-galactose, sucrose, D-lactose, melezitose, melibiose, raffinose, D-mannitol, D-sorbitol or inositol. Utilizes D-xylose, melibiose, turanose, maltose, D-fructose, D-glucose, D-lactose, sucrose, L-sorbose, trehalose, D-galactose, raffinose, L-rhamnose, L-arabinose, cellobiose, D-ribose, D-mannose, melezitose, i-erythritol, adonitol, L-arabitol, xylitol, D-sorbitol, D-mannitol, salicin, inulin, dextrin, *N*-acetyl-D-glucosamine, gluconate, lysine, amygdalin and glycerol, but not inositol, acetate, malonate or tyrosine. Resistant to streptomycin, kanamycin and polymyxin B, but sensitive to ceftazidime, chloramphenicol, erythromycin, tetracycline, ofloxacin, rifampicin, gentamicin, ampicillin and carbenicillin. The predominant fatty acids are iso-$C_{15:0}$ (32.9 %), $C_{16:0}$ (10.9 %) and summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 24.1 %). The G+C content of the DNA of the type strain is 38.5 mol%.

The type strain, SY1^T (=KCTC 22131^T=CGMCC 1.6855^T), was isolated from a soil sample from Jiangsu Province, PR China.

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