Sphingobacterium shayense sp. nov., isolated from forest soil

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A Gram-staining-negative, yellow-coloured, strictly aerobic, non-spore-forming, rod-shaped bacterium, designated HS39^T, isolated from a soil sample collected from a natural *Populus euphratica* forest in Xinjiang, China, was characterized using a polyphasic approach. The isolate grew optimally at 30–37 °C, at pH 6.5–8.0 and with 0–3 % NaCl. Analysis of the 16S rRNA gene sequence of strain HS39^T revealed that it is a member of the genus *Sphingobacterium*. *Sphingobacterium mizutaii* ATCC 33299^T was the nearest relative (94.0 % 16S rRNA gene sequence similarity). The G+C content of the genomic DNA was 40.2 mol%. The major fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The predominant isoprenoid quinone was MK-7. On the basis of phenotypic properties and phylogenetic inference, strain HS39^T represents a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium shayense* sp. nov. is proposed. The type strain is HS39^T (=CCTCC AB 209006^T =NRRL B-59203^T).

The genus *Sphingobacterium* was proposed originally by Yabuuchi *et al.* (1983), who distinguished members of the new genus from *Flavobacterium* strains by the presence of high concentrations of sphingophospholipids. At the time of writing, the genus comprised 12 species: *Sphingobacterium spiritivorum* (the type species), *S. anhuiense*, *S. antarcticum*, *S. canadense*, *S. composti*, *S. daejeonense*, *S. faecium*, *S. kitahiroshimense*, *S. mizutaii*, *S. multivorum*, *S. siyangense* and *S. thalpophilum* (Wei *et al.*, 2008; Shivaji *et al.*, 1992; Mehnaz *et al.*, 2007; Ten *et al.*, 2006; Kim *et al.*, 2006; Takeuchi & Yokota, 1992; Matsuyama *et al.*, 2008; Yabuuchi *et al.*, 1983; Liu *et al.*, 2008). The name *Sphingobacterium composti* has been proposed for two different strains (Ten *et al.*, 2006; Yoo *et al.*, 2007). As the name *Sphingobacterium composti* Yoo *et al.* 2007 is a later homonym of *Sphingobacterium composti* Ten *et al.* 2007 (http://www.bacterio.cict.fr/s/sphingobacterium.html), the type strain of *Sphingobacterium composti* Yoo *et al.* 2007 was not considered in this study. *Sphingobacterium* is the type genus of the family *Sphingobacteriaceae* (Steyn *et al.*, 1998) in the phylum *Bacteroidetes*.

In a series of studies, we attempted to isolate micro-organisms from soil samples in order to investigate the community structure using a culture-dependent method. In this study, strain HS39^T, isolated from a soil sample collected from the largest natural *Populus euphratica* forest in Xinjiang, China, located in the north-west Tarim basin (40° 56′ 35″ N 83° 22′ 18″ E), was characterized by means of a polyphasic approach to determine its taxonomic position. This approach included phylogenetic analyses based on 16S rRNA gene sequences and determination of chemotaxonomic and other phenotypic properties.

Strain HS39^T was isolated using the dilution-plating technique on Luria–Bertani (LB) agar (5 g yeast extract, 10 g peptone, 10 g NaCl, 1000 ml distilled water, pH 7.0–8.0) at 30 °C. The 16S rRNA gene of strain HS39^T was amplified by PCR with primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1527R (5′-AGAAAGGAGGTGATCCAGCC-3′). The PCR product obtained was sequenced by the dideoxynucleotide chain-termination method, using a BigDye Terminator version 3.0 cycle sequencing ready kit (Applied Biosystems) and a DNA sequencer (ABI Prism 3100). The 16S rRNA gene sequence of strain HS39^T was aligned with relevant sequences retrieved from GenBank using the CLUSTAL W program contained in the MEGA4 package. Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods, with bootstrap values based on 1000 replications (Felsenstein, 1985). Evolutionary distances were calculated using Kimura's two-parameter model (Kimura, 1980, 1983). For phylogenetic analyses, the 16S rRNA gene sequences of related type strains were obtained from the EzTaxon server (http://www.eztaxon.org; Chun *et al.*, 2007).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HS39^T is FJ816788.

Neighbour-joining phylogenetic analysis based on comparison of 1440 bp of the 16S rRNA gene sequence showed that strain HS39^T formed a coherent cluster with members of the genus *Sphingobacterium* in the phylum *Bacteroidetes* (Fig. 1). The maximum-parsimony tree showed essentially the same topology (not shown). Comparative 16S rRNA gene sequence analyses showed that strain HS39^T was closely related to *S. mizutaii* ATCC 33299^T, *S. daejeonense* TR6-04^T and *S. composti* T5-12^T, with sequence similarities of 94.0, 93.5 and 93.1 %, respectively. 16S rRNA gene sequence similarities to other members of the genus *Sphingobacterium* were lower than 93.1 %.

Strain HS39^T was cultivated routinely on LB agar or in LB broth at the same temperature for morphological, physiological and biochemical investigations. *S. mizutaii* ATCC 33299^T, *S. composti* KCTC 12578^T and *S. daejeonense* KCTC 12579^T were grown under the same conditions and used as reference strains in all physiological and biochemical tests. Cell morphology was observed using a scanning electron microscope and cell motility was studied on LB swarming agar (0.3 % agar, w/v). Gram staining, catalase and oxidase activities and hydrolysis of starch, casein, Tweens 20 and 80 and CM-cellulose were investigated as described by Smibert & Krieg (1994). Additional physiological and biochemical tests were performed by using the API 20E, API 50 CH, and API ID 32 GN kits (bioMérieux). Enzyme activities were analysed using the API ZYM kit (bioMérieux) according to the manufacturer's instructions. Growth was assessed at 4, 10, 20, 25, 28, 30, 37, 40, 42, 45 and 50 °C, at pH 5–11 (at 0.5 pH unit intervals) and with 0, 1, 2, 3, 4, 5, 6 and 7 % NaCl (w/v). Sensitivity to antibiotics was determined with the routine disc-diffusion (8 mm diameter; Sanofi Pasteur) plate method. The following antibiotics were tested: lincomycin (2 μg), ampicillin (10 μg), neomycin (30 μg), streptomycin (10 μg), tetracycline (30 μg), polymyxin B (300 IU), carbenicillin (100 μg), gentamicin (10 μg), kanamycin (30 μg), doxycycline (30 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), rifampicin (5 μg), vancomycin (30 μg), erythromycin (15 μg) and penicillin G (10 IU).

Strain HS39^T formed circular, smooth, yellow colonies after 2 days of incubation. Cells were short rods, 1.0–1.5 μm long and 0.6–0.8 μm in diameter, and were non-motile. Other phenotypic properties of strain HS39^T are given in the species description and in Table 1.

For quantitative analysis of fatty acids, strain HS39^T, *S. mizutaii* ATCC 33299^T, *S. composti* KCTC 12578^T and *S. daejeonense* KCTC 12579^T were cultivated on LB agar at 30 °C for 48 h. Preparation and analysis of fatty acid methyl esters were conducted according to the standard protocol of the MIDI Microbial Identification System (Microbial ID). Isoprenoid quinones of strain HS39^T were extracted according to the method of Collins *et al.* (1977) and analysed using reversed-phase HPLC (UltiMate 3000; Dionex) as described by Xie & Yokota (2003). Genomic DNA of strain HS39^T, *S. mizutaii* ATCC 33299^T, *S. composti* KCTC 12578^T and *S. daejeonense* KCTC 12579^T was prepared by the method of Wilson (1987) and the purity was checked spectrophotometrically. The DNA G+C contents of the four strains were determined by reversed-phase HPLC using the method of Mesbah *et al.* (1989).

Strain HS39^T contained iso-$C_{15:0}$ (28.6 %), iso-$C_{17:0}$ 3-OH (13.5 %) and summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; 37.0 %) as the major fatty acids. The fatty acid profiles of strain HS39^T and of the three reference strains were similar, although there were some differences in the proportions of some components (Table 2). The predominant isoprenoid quinone of strain HS39^T was MK-7, in line with all members of the family *Sphingobacteriaceae*, and its DNA G+C content was 40.2 mol%, within the range of values reported for *Sphingobacterium* species.

The overall phenotypic properties of strain HS39^T were consistent with its assignment to the genus *Sphingobacterium*. However, the combination of biochemical characteristics, differences in the fatty acid composition and phylogenetic inference distinguished strain HS39^T from all described *Sphingobacterium* species. Hence, it is proposed that it represents a novel species in the genus *Sphingobacterium*, with the name *Sphingobacterium shayense* sp. nov.

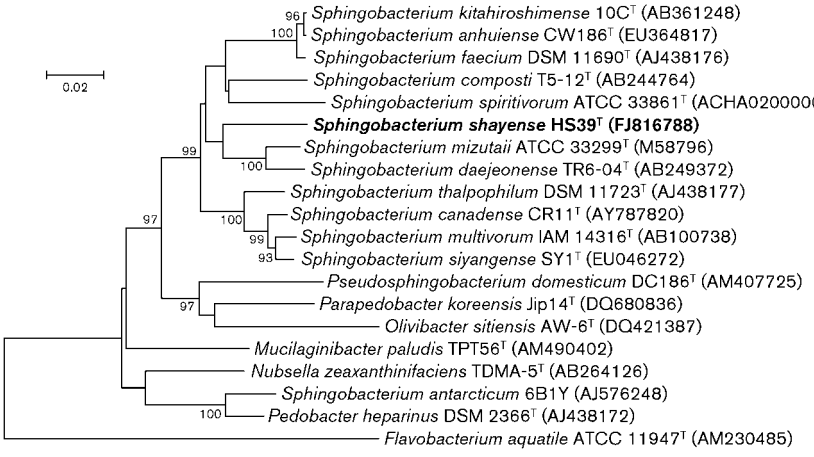

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain HS39^T and representative members of the family *Sphingobacteriaceae*. *Flavobacterium aquatile* ATCC 11947^T was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) >70 % are shown at branching points. Bar, 0.02 substitutions per nucleotide position.

Table 1. Differential characteristics of strain HS39^T and related members of the genus *Sphingobacterium*

Strains: 1, *Sphingobacterium shayense* sp. nov. HS39^T; 2, *S. mizutaii* ATCC 33299^T; 3, *S. composti* KCTC 12578^T; 4, *S. daejeonense* KCTC 12579^T. All data are from this study, including DNA G+C contents (means ± SD from three determinations). All strains were positive for catalase and oxidase activities, assimilation of D-glucose, *N*-acetylglucosamine, sucrose, maltose and salicin, acid production from glucose, melibiose and amygdalin and hydrolysis of Tween 20. All strains were negative for Gram staining, sporulation, H_2S and indole production, hydrolysis of casein and CM-cellulose, assimilation of D-ribose, inositol, glycogen, L-fucose, itaconic acid, acetate, sodium malonate, lactic acid, 3-hydroxybenzoic acid, L-serine, capric acid and 4-hydroxybenzoic acid and acid production from D-mannitol, inositol and D-sorbitol. +, Positive; −, negative; W, weakly positive.

Characteristic	1	2	3	4
Temperature range for growth (°C)	10–40	10–40	10–42	10–42
pH range for growth	5.5–9.0	5.0–9.0	6.5–9.0	5.5–9.0
Voges–Proskauer test	−	+	+	+
Hydrolysis of:				
Starch	+	+	−	−
Tween 80	+	+	+	−
Assimilation of:				
L-Rhamnose	+	−	−	−
L-Arabitol	+	−	−	−
L-Histidine	+	−	−	−
D-Sorbitol	−	+	+	−
Suberic acid	−	−	−	+
L-Proline	−	−	−	+
Acid production from:				
Sucrose	+	+	−	−
D-Galactose	+	−	+	+
D-Fructose	−	−	−	+
Melezitose	+	−	W	−
Raffinose	+	−	−	−
L-Fucose	W	+	+	−
DNA G+C content (mol%)	40.2 ± 0.1	39.9 ± 0.3	35.4 ± 0.1	37.5 ± 0.1

Description of *Sphingobacterium shayense* sp. nov.

Sphingobacterium shayense (sha.yen′se. N.L. neut. adj. *shayense* pertaining to Shaya county in Xinjiang Uyghur autonomous region of China, where the type strain was isolated).

Cells are Gram-staining-negative, non-motile, non-spore-forming, strictly aerobic rods, 1.0–1.5 μm long and 0.6–0.8 μm in diameter. After 2 days of incubation on LB agar, colonies are 1.0–2.0 mm in diameter, yellow, convex, circular and smooth with entire margins. Growth occurs between 10 and 40 °C; optimum temperature for growth is 30–37 °C. The pH range for growth is 5.5–9.0, with an optimum between pH 6.5 and 8.0. Growth occurs in the presence of 0–5 % (w/v) NaCl; optimum concentration for growth is 0–3 %. H_2S and indole are not produced. Nitrate is not reduced. The Voges–Proskauer test is negative. Catalase, oxidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase activities are present. Lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase and α-fucosidase activities are absent. Starch and Tweens 20 and 80 are hydrolysed but CM-cellulose and casein are not. L-Rhamnose, *N*-acetylglucosamine, sucrose, maltose, D-glucose, salicin, melibiose, L-arabitol and L-histidine are assimilated. D-Ribose, inositol, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, 3-hydroxybenzoic acid, L-serine, D-mannitol, L-fucose, D-sorbitol, propionic acid, capric acid, valeric acid, trisodium citrate, potassium 2-ketogluconate, 3-hydroxybutyric acid, 4-hydroxybenzoic acid and L-proline are not assimilated. Acid is produced from sucrose, D-glucose, L-rhamnose, amygdalin, D-xylose, D-galactose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, turanose, D-lyxose and L-arabitol, but not from D-mannitol, inositol, D-sorbitol, glycerol, erythritol, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, D-mannose, dulcitol, inositol, D-mannitol, D-sorbitol, inulin, glycogen, xylitol, gentiobiose, D-tagatose, D-fucose, D-arabitol, potassium

Table 2. Cellular fatty acid contents of strain HS39^T and related members of the genus *Sphingobacterium*

Strains: 1, *S. shayense* sp. nov. HS39^T; 2, *S. mizutaii* ATCC 33299^T; 3, *S. composti* KCTC 12578^T; 4, *S. daejeonense* KCTC 12579^T. Values are percentages of total fatty acids; components amounting to <1.0 % of the total fatty acids in all strains are not shown. tr, Trace (<1.0 %); −, not detected. All data are from this study.

Fatty acid	1	2	3	4
$C_{14:0}$	1.3	−	−	−
$C_{16:0}$	3.5	1.7	1.1	2.0
$C_{18:0}$	tr	1.0	1.0	1.0
$C_{16:0}$ 3-OH	2.4	tr	tr	tr
$C_{17:0}$ 2-OH	1.1	tr	tr	3.4
iso-$C_{15:0}$	28.6	38.3	38.0	32.3
anteiso-$C_{15:0}$	3.2	2.9	tr	7.8
iso-$C_{15:0}$ 3-OH	2.5	1.6	2.0	1.0
iso-$C_{17:0}$ 3-OH	13.5	18.0	17.4	15.7
iso-$C_{15:1}$ F	tr	1.8	3.0	1.6
$C_{16:1}\omega5c$	1.0	tr	tr	−
$C_{18:1}\omega5c$	tr	tr	1.7	tr
Summed feature 1*	tr	1.3	1.9	tr
Summed feature 3*	37.0	20.5	19.2	24.4
Summed feature 4*	tr	1.1	tr	tr
Summed feature 9*	tr	5.1	9.5	2.8

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 1 contained $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$ H; summed feature 3 contained $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; summed feature 4 contained anteiso-$C_{17:1}$ B and/or iso-$C_{17:1}$ I; summed feature 9 contained iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. Acid is produced weakly from melibiose, L-sorbose, L-fucose, D-arabinose, L-arabinose and D-ribose. Resistant to ampicillin (10 μg), neomycin (30 μg), streptomycin (10 μg), polymyxin B (300 IU), carbenicillin (100 μg), gentamicin (10 μg), kanamycin (30 μg) and penicillin G (10 IU). Sensitive to doxycycline (30 μg), lincomycin (2 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), rifampicin (5 μg), tetracycline (30 μg), vancomycin (30 μg) and erythromycin (15 μg). The predominant isoprenoid quinone is MK-7. The major fatty acids are iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$). The G+C content of the genomic DNA of the type strain is 40.2 mol%.

The type strain, HS39^T (=CCTCC AB 209006^T =NRRL B-59203^T), was isolated from a soil sample taken from the Xinjiang Uyghur autonomous region, PR China.

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